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# Effects of *Chlamydia Pneumoniae* Infection on Inflammation and Lipid Parameters in Humans and Mice

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Academic Dissertation to be presented with the permission of the Faculty of Medicine, University of Oulu, for public discussion in the Auditorium of Kastelli Research Center (Aapistie 1), on November 17th , 2006, at 12 noon.

National Public Health Institute

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# ABSTRACT

Since atherosclerosis is considered an inflammatory disease, there has been extensive research to determine whether microbial infections possibly contribute and maintain this inflammatory response. *Chlamydia pneumoniae*, a common causative agent of respiratory tract infections, was associated with cardiovascular diseases as early as the late 80´s. *C. pneumoniae* is a gram-negative bacterium which resembles viruses in terms of obligate intracellular growth. *C. pneumoniae* is able to infect several cell types, including circulatory monocytes, and by using monocytes as a vehicle, *C. pneumoniae* can disseminate from the lungs into extrapulmonary sites, including the subendothelial space of the arterial wall. In addition to inflammation, lesion development in the arterial wall requires macrophage transformation to lipid-laden foam cells in the presence of an atherogenic cholesterol source like low-density lipoprotein (LDL) cholesterol. Chronic *C. pneumoniae* infection has been demonstrated to change lipoprotein distribution towards a pro-atherogenic lipid profile with increased VLDL and LDL and decreased high-density lipoprotein (HDL). Statins, a class of cholesterol drugs currently in wide use, not only decrease LDL and increase HDL cholesterol levels but also have an anti-inflammatory effect and are thus highly cardioprotective.

The present study investigated the effect of acute *C. pneumoniae* infection on serum lipid metabolism in mice. In addition, the effect of pravastatin treatment on *C. pneumoniae* infection and the infection-induced pulmonary inflammatory response and changes in serum lipid metabolism were investigated in an NIH/S mouse model during regular chow and a high fat diet. The second aim was to develop a new diagnostic method for *C. pneumoniae* infection and apply the method to analyze clinical samples.

In NIH/S mice with regular chow, acute *C. pneumoniae* infection had mild effects on serum lipids and caused a transient yet significant increase in serum triglycerides and a decrease in lecithin-cholesterol acyltransferase (LCAT) activity. However, these changes, if prolonged during chronic *C. pneumoniae* infection, may impair lipoprotein metabolism and produce a pro-atherogenic lipid profile. One unexpected observation was that pravastatin treatment during a high fat diet induced a

significant increase primarily in triglycerides but not in cholesterol both in *C. pneumoniae*-infected and uninfected mice. Pravastatin accelerated the pulmonary inflammatory response to the *C. pneumoniae* infection, but had no effect on infection load. Since NIH/S mice normally have an antiatherogenic lipid profile with high HDL and low apoB-containing lipoproteins (VLDL, LDL), mouse strains or other animal models with a more humanized lipid profile (high LDL, low HDL) should be used in order to detect the favorable effects of statins on serum lipid levels. Since anti-inflammatory and cholesterol-lowering capacities differ among various statins, other statins might be more effective during *C. pneumoniae* infection.

To develop a sensitive and specific enzymeimmunoassay (EIA) method for the measurement of serum chlamydial lipopolysaccharide (cLPS) concentration, a novel approach was employed. LPS was dissociated and captured from lipoprotein and immune complexes in serum by lipopolysaccharide binding protein (LBP) in the presence of an octylglucoside detergent. The method was applied to the quantitation of cLPS in the sera of patients with acute coronary syndrome (ACS) and their controls. Significantly higher cLPS concentrations and prevalence were found in ACS patients compared to their controls. During ACS, the highest cLPS levels were found within 48 hours and the cLPS levels rapidly decreased as early as one week after the event. cLPS levels were significantly higher in acute myocardial infarction (AMI) patients as compared to patients with unstable angina (UA) at the time of hospitalization. In addition, a significant correlation was demonstrated between serum cLPS and highly sensitive C-reactive protein (hsCRP) levels throughout the one-year follow-up period in ACS patients. Our data not only strengthen the association between *C. pneumoniae* infection and cardiovascular disease but also highlight the role of *C. pneumoniae* LPS in the inflammatory process during acute coronary syndrome.

Keywords: *Chlamydia pneumoniae*, NIH/S mouse model, lipid metabolism, chronic infection, acute coronary syndrome, lipopolysaccharide, highly sensitive C-reactive protein

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# TIIVISTELMÄ

Koska ateroskleroosia pidetään nykyään tulehdussairautena, viime vuosien aikana on tutkittu voivatko mikrobi-infektiot aiheuttaa ja ylläpitää tätä tulehdusreaktiota. *Chlamydia pneumoniae*, keuhkoklamydia, on yleinen hengitystieinfektioiden aiheuttaja, joka jo 1980-luvun lopulla yhdistettiin yhdeksi sydän- ja verisuonitautien riskitekijäksi. Keuhkoklamydia on gram-negatiivinen bakteeri, joka virusten tavoin lisääntyy ainoastaan elävien solujen sisällä. Keuhkoklamydia infektoi useita erilaisia solutyyppejä ja sen leviäminen keuhkojen ulkopuolelle, kuten valtimon seinämään, tapahtuu infektoitujen monosyyttien välityksellä. Tulehduksen lisäksi valtimovaurion muodostuminen edellyttää makrofagien muuntumista kolesterolirikkaiksi vaahtosoluiksi aterogeenisen kolesterolilähteen, LDL:n, läsnäollessa. Kroonisen keuhkoklamydiainfektion on osoitettu liittyvän verenkierron pro-aterogeeniseen lipidiprofiiliin mikä ilmenee kohonneena LDL- ja alentuneena HDL-kolesterolipitoisuutena. Nykyisin laajasti käytetyt kolesterolilääkkeet, statiinit, eivät ainoastaan laske LDL kolesterolia ja nosta HDL tasoja, vaan ovat myös tulehdusta estäviä ja suojaavat siten tehokkaasti sydän- ja verisuonitaudeilta.

Tässä tutkimuksessa analysoitiin akuutin keuhkoklamydiainfektion vaikutusta seerumin rasva-aineenvaihduntaan. Lisäksi selvitettiin pravastatiinin vaikutusta keuhkoklamydiainfektioon sekä infektion indusoimaan keuhkojen tulehdusvasteeseen sekä seerumin lipidimuutoksiin normaalilla ja rasvapitoisella rehulla ruokituilla NIH/S hiirillä. Tarkoituksena oli myös kehittää uusi menetelmä keuhkoklamydiainfektion diagnotiikkaan ja soveltaa tätä menetelmää potilasaineistojen tutkimiseen.

Keuhkoklamydiainfektio aiheutti lieviä muutoksia seerumin rasvatasoihin NIH/S hiirillä: havaittiin vain ohimenevä mutta merkittävä triglyseriditasojen nousu ja lesitiini-kolesteroli asyylitransferaasientsyymin (LCAT) aktiivisuuden lasku. Jos nämä muutokset pitkittyvät kroonisen keuhkoklamydiainfektion aikana, ne voivat häiritä lipoproteiinien aineenvaihduntaa ja johtaa sydäntauteja edistävän pro-aterogeenisen lipidiprofiilin syntyyn verenkierrossa. Yllättävä havainto oli, että pravastatiinihoito rasvaruokituilla hiirillä aiheutti merkittävän nousun triglyserideissä mutta ei kolesterolissa. Samantyyppinen vaste nähtiin pravastatiinihoidetuilla

hiirillä keuhkoklamydiainfektion aikana. Pravastatiinihoito aikaisti, mutta ei vähentänyt keuhkojen tulehdusvastetta. Koska NIH/S hiirillä on sydäntaudeilta suojaava rasvaprofiili, korkea HDL ja matalat apoB-lipoproteiinit (LDL, VLDL), statiinihoidon suotuisat vaikutukset saattaisivat tulla esiin hiirillä tai muilla eläimillä, joilla on "ihmistyyppinen" seerumin lipoproteiiniprofiili (korkea LDL, matala HDL). Koska tulehdusta estävä ja kolesterolia alentava vaikutus vaihtelee eri statiinien välillä, toiset statiinit saattaisivat olla tehokkaampia.

Sovelsimme uutta lähestymistapaa kehittäessämme herkkää ja spesifistä entsyymi-immunologista määritysmenetelmää klamydian lipopolysakkaridin (cLPS) mittaamiseen seerumista. LPS irroitettiin seerumin proteiinikomplekseista lipopolysakkaridia sitovan proteiinin (LBP) avulla oktyyliglukosidi-detergentin läsnäollessa. Kun menetelmää sovellettiin klamydian LPS:n mittaamiseen akuutti koronaarisyndroomapotilailta ja heidän kontrolleiltaan, havaittiin että potilaiden seerumeissa oli kontrolleja useammin klamydian LPS:a ja lisäksi LPS:n pitoisuus potilailla oli myös merkittävästi korkeampi kuin kontrolleilla. Korkeimmat klamydian LPS-pitoisuudet havaittiin 48 tunnin kuluessa akuutin sydänkohtauksen alkamisesta, ja LPS-tasot olivat jo viikon kuluttua tapahtumasta laskeneet merkittävästi. Sairaalaan tulohetkellä klamydian LPS-taso oli merkittävästi korkeampi akuutin sydäninfarktin saaneilla kuin epästabiilia rintakipua sairastavilla potilailla. Lisäksi klamydian LPS:n tasot korreloivat merkittävästi herkän C-reaktiivisen proteiinin tasoihin (hsCRP) läpi vuoden kestävän seuranta-ajan. Nämä tulokset eivät ainoastaan vahvista keuhkoklamydiainfektion yhteyttä sydäntauteihin, vaan ne myös viittaavat siihen, että klamydia-LPS osaltaan aiheuttaa akuuttiin sydänkohtaukseen liittyvän tulehdusreaktion.

Avainsanat: keuhkoklamydia, hiirimalli, rasva-aineenvaihdunta, krooninen infektio, akuutti koronaarisyndrooma, lipopolysakkaridi, herkkä C-reaktiivinen proteiini

# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| ABCA1 | ATP-binding cassette transporter A1 |
| ACS | acute coronary syndrome |
| AMI | acute myocardial infarction |
| apo | apolipoprotein |
| APOE KO | apolipoprotein E knockout |
| APR | acute phase response |
| ATP | adenosine triphosphate |
| AUC | area under the curve |
| BPI | bacteridical permeability increasing protein |
| CETP | cholesterol ester transfer protein |
| CF | complement fixation |
| CHD | coronary heart disease |
| CM | chylomicron |
| COMC | chlamydial outer membrane complex |
| EB | elementary body |
| EIA | enzyme immunoassay |
| EM | electron microscopy |
| HDL | high density lipoprotein |
| HL | hepatic lipase |
| HMG-CoA | 3-hydroxy-3-methylglutaryl coenzyme A |
| hsCRP | highly sensitive C-reactive protein |
| Hsp | heat schock protein |
| ICC | immunocytochemistry |
| IDL | intermediate density lipoprotein |
| IFN | interferon |
| IFU | inclusion forming unit |
| IL | interleukin |
| IM | immunofluorescence |
| Inc | inclusion membrane protein |
| iNOS | inducible nitric oxid synthase |
| kDa | kilodalton |
| KDO | 3-deoxy-D-manno-octulosonate |

| | |
|---|---|
| LBP | lipopolysaccharide binding protein |
| LCAT | lecithin-cholesterol aclyltransferase |
| LDL | low density lipoprotein |
| LDLR | LDL receptor |
| LDLR KO | low density lipoprotein receptor knockout |
| LPL | lipoprotein lipase |
| LPS | lipopolysaccharide |
| LRP | LDL-receptor related protein |
| mCD14 | membrane bound CD14 |
| MCP | monocyte chemoattractant protein |
| MIF | microimmunofluorescence |
| MOMP | major outer membrane protein |
| NacGlc | N-acetyl-D-glucosamine |
| NF-κβ | nuclear factor κβ |
| NIH mouse | National Institute of Health mouse |
| OM | outer membrane |
| Omp | Outer membrane protein |
| PAF-AH | platelet activating factor acetylhydrolase |
| PBMC | peripheral blood mononuclear cell |
| PCR | polymerase chain reaction |
| p.i. | post infection |
| PLTP | phospholipid transfer protein |
| Pmp | polymorphic membrane protein |
| PON | paraoxonase |
| RB | reticulate body |
| ROC-curve | receiver operating characteristic curve |
| SAA | serum amyloid A |
| sCD14 | soluble CD14 |
| SREBP | sterol regulatory binding protein |
| TLR | Toll-like receptor |
| TNFα | tumor necrosis factor α |
| TTS | type III secretion |
| VLDL | very low density lipoprotein |
| VSMC | vascular smooth muscle cells |

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Tiirola T., Erkkilä L., Laitinen K., Leinonen M., Saikku P., Bloigu A. and Jauhiainen, M. Effect of acute *Chlamydia pneumoniae* infection on lipoprotein metabolism in NIH/S mice. Scand J Clin Lab Invest 2002; 62: 477-484

II Tiirola T., Erkkilä L., Bloigu A., Haasio K., Laitinen K., Saikku P., Leinonen M. and Jauhiainen M. Effect of pravastatin treatment on *Chlamydia pneumoniae* infection, inflammation and serum lipids in NIH/S mice. Submitted

III Tiirola T., Jaakkola A., Bloigu A., Paldanius M., Sinisalo J., Nieminen M.S., Silvennoinen-Kassinen S., Saikku P., Jauhiainen M. and Leinonen M. Novel enzymeimmunoassay utilizing lipopolysaccharide-binding protein as a capture molecule for the measurement of chlamydial lipopolysaccharide in serum. Diagn Microb Inf Dis 2006, 54:7-12

IV Tiirola T., Sinisalo J., Nieminen M.S., Silvennoinen-Kassinen S., Paldanius M., Jauhiainen M., Saikku P. And Leinonen M. Chlamydial lipopolysaccharide is present in serum during acute coronary syndrome and correlates with CRP levels. Atherosclerosis, in press



In addition, some unpublished data are presented.

# 1 INTRODUCTION

Atherosclerosis is a leading cause of mortality in Western industrialized countries. Hypertension, hypercholesterolemia [high low density lipoprotein (LDL) and low high density lipoprotein (HDL) cholesterol levels], cigarette smoking, diabetes, obesity, physical inactivity (Braunwald, 1997), age (Gordon *et al.*, 1977; Uren *et al.*, 1995), psychosocial (Eaker *et al.*, 1992) and genetic (Schildkraut *et al.*, 1989) factors as well as male gender (Jousilahti *et al.*, 1999) are the well-established risk factors for coronary heart disease (CHD). However, all CHD cases cannot be explained by the preceding risk factors only. Since infection and inflammation have been appreciated as relevant underlying risk factors for atherosclerosis during the past decade (Ross, 1999), interest in the search for candidate pathogens has increased.

Certain pathogens, such as *Chlamydia pneumoniae*, cytomegalovirus, herpes simplex virus, *Porphyromonas gingivalis*, and *Helicobacter pylori*, are currently linked with the pathogenesis of CHD and atherosclerosis (Fong, 2000; Leinonen *et al.*, 2002; Saikku *et al.*, 1988). Among these agents, the role of *C. pneumoniae* in atherosclerotic diseases has been studied most intensively and the association has been verified seroepidemiologically, by the presence of viable organisms in the lesions as well as by animal and some clinical studies. The earliest findings were based on the presence of seroresponse in acute myocardial infarction (AMI) and higher *C. pneumoniae* antibody titers in CHD patients than in their controls (Saikku *et al.*, 1988), and, at present, there is mounting evidence linking *C. pneumoniae* infection with atherosclerosis (Grayston *et al.*, 1995; Kuo *et al.*, 1993a; Kuo *et al.*, 1995a; Kuo *et al.*, 1993b; Muhlestein *et al.*, 1996; Ong *et al.*, 1996; Ramirez, 1996; Shor *et al.*, 1992). The presence of *C. pneumoniae* has been demonstrated both in circulating monocytes and within atherosclerotic lesions (Belland *et al.*, 2004) and even viable *Chlamydiae* have been isolated from the atherosclerotic plaques and abdominal aortic aneurysms (Johnston *et al.*, 2001; Karlsson *et al.*, 2000; Maass *et al.*, 1998; Ramirez, 1996).

*C. pneumoniae*, a small, obligatory intracellular gram-negative bacterium is one of the most common respiratory pathogens worldwide (Grayston *et al.*, 1990). Seroepidemiological surveys indicate that about 50 % of middle-aged adults have *C. pneumoniae* antibodies and that reinfections are common (Grayston *et al.*, 1990). The clinical features of infections that are transmitted via airways from person to person vary from mild, even asympomatic upper respiratory tract infections to severe bronchitis and pneumonia (Saikku *et al.*, 1985; Thom *et al.*, 1990). As with all chlamydial species, *C. pneumoniae* has a tendency to cause persistent and chronic infections, which are suggested to connive in the development of reactive arthritis

(Braun *et al.*, 1994), erythema nodosum (Erntell *et al.*, 1989; Saikku *et al.*, 1985), sarcoidosis (Ekman *et al.*, 1993a; Grönhagen-Riska *et al.*, 1988), asthma (Hahn, 1992), chronic obstructive pulmonary disease (Von Hertzen *et al.*, 1997) and atherosclerosis (Belland *et al.*, 2004; Leinonen *et al.*, 2002).

Because of serious implications for a variety of diseases, eradication of *C. pneumoniae* infection has become very important. However, screening for *C. pneumoniae* infection requires reliable diagnostic methods. Currently, laboratory diagnosis of *C. pneumoniae* infection is based on serology, cultivation or demonstration of the presence of the organism by nucleic acid amplification tests such as polymerase chain reaction (PCR). However, serology is only an indirect indicator of current or past infection. Representative samples can be impossible to obtain from the diseased tissues, e.g. from atherosclerotic plaques for the demonstration of the organisms. In addition, the methods may lack specificity (serology), sensitivity (culture) or both (PCR). Therefore, there exists an urgent need for new sensitive and specific diagnostic tools especially for the diagnosis of chronic *C. pneumoniae* infections.

# 2 REVIEW OF THE LITERATURE

## *2.1 Chlamydia pneumoniae*

### 2.1.1 Taxonomy

*Chlamydia pneumoniae* is a member of genus *Chlamydia*, which belongs to the family *Chlamydiaceae* in the order *Chlamydiales* (Moulder *et al.*, 1984). *C. pneumoniae*, first considered to be an unusual *C. psittaci* strain called TWAR (Saikku *et al.*, 1985), was identified as a third chlamydial species in the late 1980s (Grayston *et al.*, 1990) and was followed by *C. pecorum*, a pathogen of ruminants (Fukushi *et al.*, 1992).

Based on phylogenetic analyses of the 16S and 23S rRNA genes, a new taxonomic classification was recommended in 1999 (Everett *et al.*, 1999). Accordingly, the genus *Chlamydia* should be broken into two genera, *Chlamydia* and *Chlamydophila* (Table 1). Furthermore, three novel families, *Parachlamydiaceae, Simniaceae and Waddliaceae*, were suggested to be included in the genus *Chlamydia* (Everett *et al.*, 1999). Nonetheless, the new taxonomy is still under debate and lacks consensus among chlamydial authorities (Schachter *et al.*, 2001).

***Table 1. Proposal for Chlamydial taxonomy (modified from Everett et al., 1999).***

| Genus | Species | Host | Route of entry |
|---|---|---|---|
| *Chlamydia* | *C. trachomatis* | Human | Pharynx, eye, genital, rectal |
| | *C. suis* | Swine | Pharynx |
| | *C. muridarum* | Mouse, hamster | Pharynx, genital |
| *Chlamydophila* | *C. psittaci* | Bird | Pharynx, eye, genital |
| | *C. pneumoniae* | Human | Pharynx, eye |
| | *C. pecorum* | Mammals | Oral |
| | *C. felis* | Cat | Pharynx, eye, genital |
| | *C. caviae* | Guinea pig | Pharynx, eye, genital, urethral |
| | *C. abortus* | Mammals | Oral, genital |

### 2.1.2 History

Chlamydial diseases have been known since antiquity (Oriel, 1994), although the pathogen itself remained mysterious for a long time. The earliest chlamydial species, *C. trachomatis*, perceived as a trachoma agent in 1907, was first considered protozoa and thus conferred the name *Chlamydozoaceae* (Ward, 1983). Based on the small size and poor growth in culture media *Chlamydia* was later categorized as an unusual virus. In 1930s, Bedson & Bland described a second chlamydial species, *C. psittaci*, as an "obligate intracellular bacteria-like parasite", an assumption that was not attested until the 1960s.

*C. pneumoniae* was originally discovered in the 1940s by Joseph Smadel, who described atypical psittacosis in patients without bird contact. This "humanized strain of virus" appeared to disseminate from human to human via the respiratory route (Smadel, 1943). Several epidemics of this type as arnithosis or "para-ornithosis" were described from Scandinavia in the 1950's (Jansson, 1960). However, *C. pneumoniae* needed to be "re-discovered" in the 1980's (Saikku *et al.*, 1985) and was named TWAR after two laboratory isolates TW-183 (isolated from the child eye in Taiwan in 1965) and AR-39 (isolated from a throat swab of a student in USA in 1983) (Grayston *et al.*, 1986). Genetically, *C. pneumoniae* shares less than 10 % sequence identity with other chlamydial species (Grayston, 1989).

### 2.1.3 Structure

Chlamydial envelope components play a crucial role in the infection process, i.e. protecting extracellular elementary bodies (EBs) from the action of antibodies, involvement in the attachment and uptake of the EBs to the host cell, and presumably also preventing lysosomal fusion after EB entry into the host cell (Longbottom *et al.*, 1998).

Both extra and intracellular chlamydial forms have a double cell membrane, which is peculiar to all gram-negative bacteria. The inter-membrane space lacks muramic acid (Barbour et al., 1982; Fox et al., 1990; Garrett et al., 1974), which is an essential polymer of the peptidoglycan layer in the bacterial cell wall. The rigidity of the cell wall is chiefly maintained by disulfide-bridges in the cysteine-rich major outer membrane protein (MOMP) (Campbell et al., 1990), which covers about 60 % of the chlamydial outer membrane (OM) mass (Caldwell et al., 1981). *C. pneumoniae* MOMP is also surface-exposed and immunogenic; it is approximately 42 kilodalton (kDa) in size (Campbell et al., 1990; Knudsen et al., 1999; Wolf et al., 2001). Serovar-, subspecies and species-specific differences in *C. trachomatis*

MOMP have been identified (Conlan et al., 1988; Stephens et al., 1988; Zhang et al., 1989), whereas *C. pneumoniae* MOMP appear constant (Perez Melgosa et al., 1991).

In *Chlamydiae*, MOMP is a major protein component in the chlamydial outer membrane complex (COMC). COMC also contains other cysteine-rich proteins, outer membrane proteins (Omps). Omp2 and Omp3 in *C. psittaci* are not surface-exposed (Everett *et al.*, 1995), while Omp 4 and Omp 5 have been localized by immunofluorescence (IM) microscopy on the surface of *C. pneumoniae* (Knudsen *et al.*, 1999).

Quite recently, a new family of chlamydial proteins, polymorphic membrane proteins (Pmps), was indentified in the chlamydial genome. Even 21 Pmps have been found in *C. pneumoniae* but only 9 Pmp genes in *C. trachomatis* genome, respectively (Kalman *et al.*, 1999). The signal peptide sequence indicates the localization of most Pmps on the outer membrane in *C. pneumoniae* (Pedersen *et al.*, 2001). The definite function of Pmps is not known, but they have been proposed to act as primary mediators in pathogen-host cell interactions (Christiansen *et al.*, 1999).

Heat schock proteins, Hsps, are conserved proteins among prokaryotes and eukaryotes and their expression can be induced by stress or injury, like infections (Kaufmann, 1990). *Chlamydia* express Hsps under stress conditions, for instance during a deficiency of tryptophan, the amino acid that *Chlamydia* is unable to synthesize (Beatty *et al.*, 1994a; Beatty *et al.*, 1994b). To date, three chlamydial Hsps, cHsp10, cHsp60 and cHsp70, have been characterized (LaVerda *et al.*, 1997; Morrison *et al.*, 1989; Raulston *et al.*, 1993). Chlamydial Hsp60, produced during the growth cycle and released from inclusions during the host cell lysis, induces the production of inflammatory mediators by vascular smooth muscle cells, endothelial cells and macrophages (Kol *et al.*, 1999; Kol *et al.*, 1998). A host immune response to microbial Hsp60 may trigger an autoimmune response against human Hsp60 and eventually speed up the progression of atherosclerosis (Kol *et al.*, 1998; Mosorin *et al.*, 2000; Wick *et al.*, 2001).

There are also proteins localized solely in chlamydial inclusion bodies, termed inclusion membrane (Inc) proteins. Inc proteins are secreted to be part of the inclusion membrane by the type III secretion (TTS) apparatus (Subtil *et al.*, 2000). Over 40 candidate sequences for Inc have been hitherto identified in the genomes of *C. trachomatis* and *C. pneumoniae* comprising about 3 % and 6 % of the whole genome, respectively (Bannantine *et al.*, 2000). The first Inc proteins, IncA, IncB and IncC were found in the *C. psittaci* inclusion membrane (Bannantine *et al.*, 1998; Rockey *et al.*, 1995). Thereafter four additional Incs, IncD-G were discovered in *C. trachomatis* (Scidmore-Carlson *et al.*, 1999). So far, over 40 Inc proteins in *C. trachomatis* and over 60 Incs in *C. pneumoniae* have been identified (Bannantine *et al.*, 2000). The function of the Incs is not clear, but they may be involved in contact

with the cytosol of the host cell, e.g. in lipid trafficking from the Golgi region to the chlamydial inclusion (Bannantine *et al.*, 2000).

An additional set of 24 proteins that are TTS substrates but do not belong to the Inc family have been identified most recently in *C. pneumoniae*, *C. trachomatis* and *C. caviae*. At least one of these newly characterized proteins translocates during proliferation from the chlamydial inclusion to host cell cytoplasm (Subtil *et al.*, 2005). TTS proteins probably induce host cell responses to ensure favorable conditions for chlamydial growth (Subtil *et al.*, 2004).

### Lipopolysaccharide (LPS)

All gram-negative bacteria carry a unique lipopolysaccharide (LPS, endotoxin) as a major constituent of the outer membrane. LPS is a glycolipid compound comprised of three main structural domains: hydrophobic lipid A moiety, hydrophilic core oligosaccharide and O-polysaccharide moieties (Fig. 1). O-polysaccharide, also termed O-antigen, is generally composed of 20 to 40 repeating sugar units, each of which comprises five sugar molecules. O-polysaccharide is the outermost part of LPS. The core oligosaccharide, which lies between the O-polysaccharide and lipid A moieties, is subdivided into an inner and outer core (Fig. 1). Rough LPS types without O-polysaccharide are denoted Ra-Re forms according to their core oligosaccharide composition (Fig. 1).

The membrane anchor of LPS is called Lipid A, which constitutes an endotoxically active region of LPS (Seydel, 1998). The structure of lipid A in various bacteria is relatively conserved, sharing 1, 4′-bisphosphorylated-β1, 6-linked N-acetyl-D-glucosamine (NacGlc) disaccharide, which is acylated by four (R)-3-hydroxy fatty acids (of about 12-14 carbon atoms). The fifth fatty acid is branched from the 3-hydroxy fatty acid, which is acylated in glucosamine, whereas the location of the sixth fatty acid may differ (Rietschel *et al.*, 1994; Zahringer *et al.*, 1994). Structural variations in lipid A are mainly found in the acylation pattern (Rietschel *et al.*, 1996).

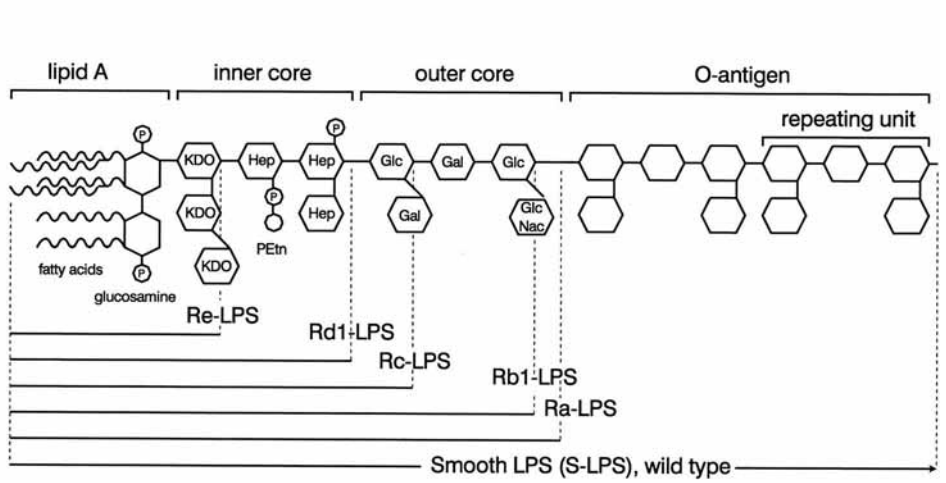


***Fig. 1. LPS structure.*** P, phosphate; KDO, 3-deoxy-D-*manno*-octulosonic acid; Hep, L-*glycero*-D-*manno*-heptose; Etn, ethanolamine; PEtn, phosphatidyl-ethanolamine, Glc, D-glucose; Gal, D-galactose, GlcNac, N-acetyl-D-glucosamine

Chlamydial LPS (cLPS) is a well-characterized, surface-exposed antigen located on the outer membrane of *Chlamydia*. The cLPS is truncated, lacking O-antigen, and is thus a phenotypically rough LPS (Nurminen *et al.*, 1983). However, an LPS smooth-rough phase variation in *C. psittaci* has been described (Lukacova *et al.*, 1994). The cLPS consists of pentasaccharide containing lipid A moiety, which is covalently linked to a unique linear 3-deoxy-D-*manno*-octulosonic acid (KDO)-trisaccharide (Fig. 2.) (Brade *et al.*, 1987). Quite recently, a new KDO-tetrasaccharide has been described for *C. psittaci* (Muller-Loennies *et al.*, 2005). Although the lipid A structure among gram-negative bacteria is rather similar, there are some essential structural variations in the cLPS lipid A moiety. Acyl chains of cLPS lipid A are unusually long, composing over 20 carbon atoms. Further, there is amide-linkage between the 3-hydroxy fatty acids (Brade *et al.*, 1986; Nurminen *et al.*, 1985). To date, the structures of the LPS of *C. trachomatis* serotypes $L_2$ (Rund *et al.*, 1999) and E (Heine *et al.*, 2003) as well as *C. psittaci* 6BC strain (Rund *et al.*, 2000) and *C. pneumoniae* Kajaani 6 strain (Hussein *et al.*, 2001) have been elucidated.

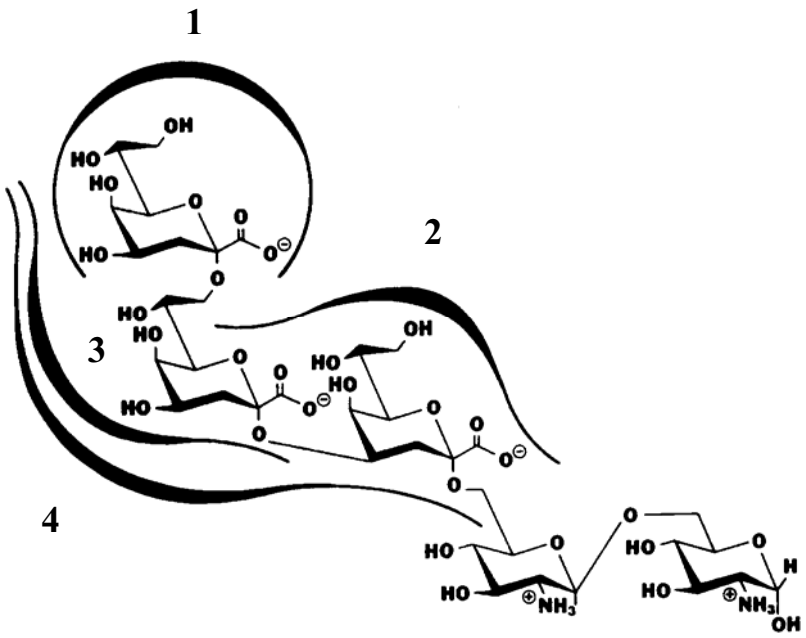


***Fig. 2. Structure of core oligosaccharide in Chlamydiae (modified after Brade et al., 1994).*** Monoclonal antibodies produced against epitopes 3 and 4 are *Chlamydia*-specific.

LPSs are considered a major antigen in gram-negative bacteria (Luderitz *et al.*, 1966a; Luderitz *et al.*, 1966b). LPSs can elicit a variety of pathophysiological responses, including fever, coagulant activity, and septic chock (Parrillo, 1993; Rietschel *et al.*, 1992; Ulevitch *et al.*, 1995). LPSs may also activate an acute inflammatory response by inducing the production of various inflammatory cytokines, such as tumor necrosis factor (TNF) $\alpha$ and interleukin (IL) 1$\beta$ (Morrison *et al.*, 1987; Raetz, 1990; Rietschel *et al.*, 1992).

Yet, cLPS is less effective than e.g. enterobacterial LPS in eliciting inflammatory a cytokine response in serum (Ingalls *et al.*, 1995; Netea *et al.*, 2002). Nonetheless, cLPS is capable of inducing macrophage foam cell formation in the presence of exogenous LDL (Kalayoglu *et al.*, 1998a). The diminished biological activity of cLPS may be due to structural divergences, i.e. i) an unusual acylation pattern (five

acyl groups instead of six) ii) long fatty acid chains and iii) fewer hydroxy fatty acids attached to the glucosamine disaccharide (Qureshi *et al.*, 1997). Indeed, the LPS of *C. trachomatis* L2 displays up to 100 to 1000-fold less potency than the LPS of *E. coli* O55:B5 to induce TNFα secretion (Prebeck *et al.*, 2003). In serum-free conditions, however, cLPS is a more potent inducer of TNFα production than enterobacterial (*E. coli* O158:B8) LPS. Since interactions with LPS-carrier proteins, e.g. LPS binding protein (LBP), are mediated by the lipid A part of LPS, it has been suggested that i) divergence in cytokine production is mainly due to structural differerences in the lipid A (Kalayoglu *et al.*, 2000) and ii) LPS in serum-free conditions interacts more efficiently with an LPS receptor on a monocyte surface and is transferred into the cell by the unknown mechanism. Although *in vitro* studies suggest the role of cLPS in atherogenesis, further studies are needed to confirm the biological mechanism of cLPS in this process.

### 2.1.4 Developmental cycle

*Chlamydiae* as obligate intracellular parasites of eukaryotic cells have a unique developmental cycle with two distinct morphological forms: extracellular, infective, metabolically inactive elementary body (EB) and intracellular, metabolically active multiplying reticulate body (RB). Elementary bodies (EBs) are small, approximately 0.3 μm in diameter and have an electron-dense nucleoid. RBs are substantially larger, from 1 to 1.5 μm in diameter having an equally dispersed, reticulate cytoplasm (Friis, 1972). *Chlamydiae* have traditionally been considered energy parasites; as incapable of producing the energy needed for replication, they are dependent on adenosine triphosphate (ATP) derived from the host cell (Hatch *et al.*, 1982). Surprisingly, ATPase genes have been found in chlamydial genome (Stephens *et al.*, 1998), indicating ATP-producing capacity, which may be important in the early and late stages of the developmental cycle, when host cell ATP is not available (Hatch *et al.*, 1982).

The first description of the chlamydial growth cycle was based on electron microscopic studies of *C. trachomatis* and *C. psittaci* in cell culture (Higashi, 1965). Details in the cycle diverge between the chlamydial strains and host cell involved (Higashi, 1965; Storz *et al.*, 1977; Vanrompay *et al.*, 1996). The cycle is initiated by the endocytosis of an EB by a target host cell (Fig.3, A). After inhibition of phagolysosomal fusion (B), the EBs differentiate to the RBs (C). RBs replicate by binary fission inside the inclusion vesicles derived from the host cell lipids (D). Persistent aberrant chlamydial forms may develop at this phase under stress conditions (e.g. interferon (IFN) γ exposure, penicillin, iron deprivation). Abnormal RBs

no not divide nor mature into EBs. Without stress, RBs differentiate back to EBs (E), the host cell is lysed, and EB forms are released to initiate a new round (F).

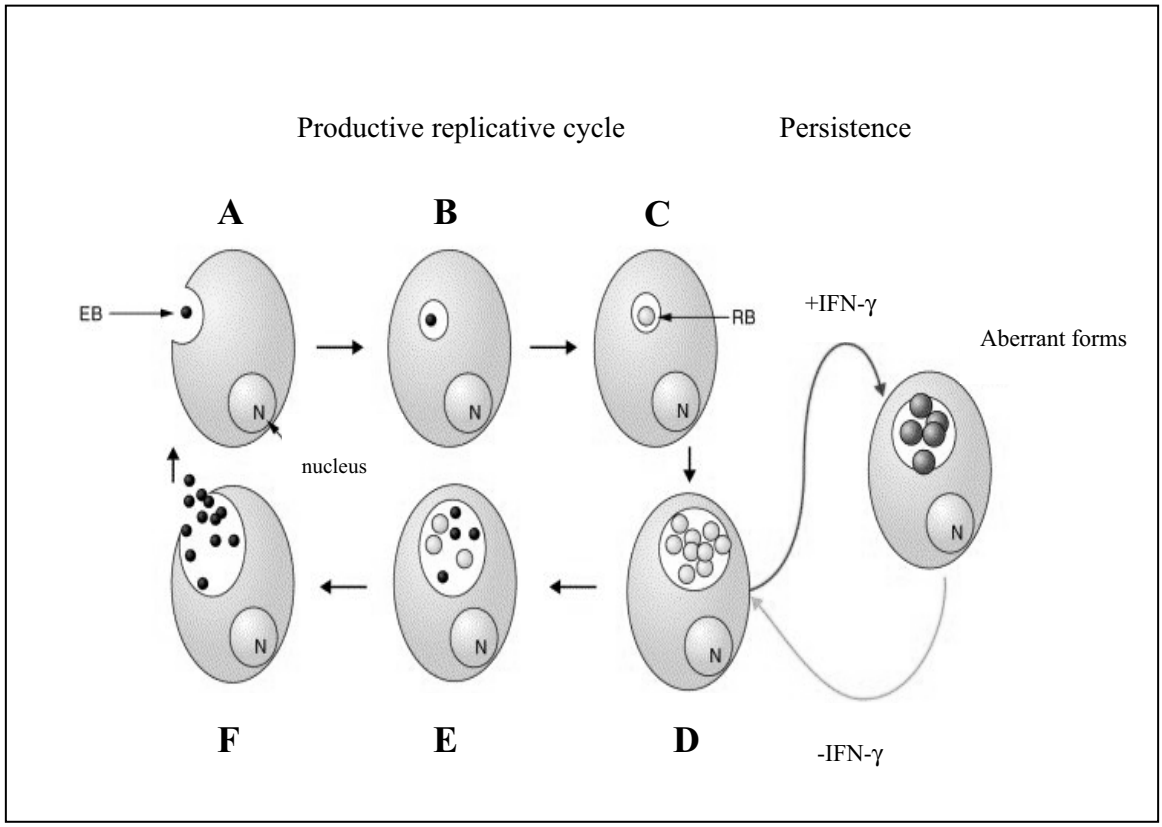


***Fig. 3. Developmental life cycle in Chlamydia (modified after Rottenberg et al., 2002).***

## 2.1.5 Pathogenesis

*C. pneumoniae* survives and multiplies within the cell types found in a vascular atherosclerotic lesion including endothelial cells, smooth muscle cells, monocytes, macrophage-foam cells as well as T-lymphocytes (Airenne *et al.*, 1999; Gaydos *et al.*, 1996; Godzik *et al.*, 1995; Haranaga *et al.*, 2001; Kaukoranta-Tolvanen *et al.*, 1994). The organism induces the production of several proinflammatory cytokines and inflammatory mediators, such as monocyte chemoattractant protein (Grube *et*

*al.*) 1, IL-8, TNFα and IL-1β (Jahn *et al.*, 2000; Kaukoranta-Tolvanen *et al.*, 1996; Krull *et al.*, 2004; Molestina *et al.*, 2000). *In vitro*, the adherence and attachment of monocytes to aortic endothelial cells is accelerated by *C. pneumoniae* (Kalayoglu *et al.*, 2001). In addition, *C. pneumoniae*-infected monocyte-derived macrophages accumulate cholesterol esters and form cholesterol-loaded foam cells in the presence of LDL (Kalayoglu *et al.*, 1998b).

Monocytes have been suggested to serve as transport vehicles in the dissemination of *C. pneumoniae* to extrapulmonary organs. Animal studies have clearly demonstrated that *C. pneumoniae* can spread from the lungs to other sites (Campbell *et al.*, 1998) including aortic tissue (Fong *et al.*, 1997; Hu *et al.*, 1999; Moazed *et al.*, 1997). The migration of *C. pneumoniae*-infected alveolar macrophages from the lungs into the bloodstream via the lymphatic route has been postulated to be involved in mice (Moazed *et al.*, 1998) and rabbits (Gieffers *et al.*, 2004). Supporting this, several human studies have demonstrated circulatory *C. pneumoniae* DNA in peripheral blood mononuclear cells (PBMC) in patients with cardiac disease more frequently than in healthy controls (Boman *et al.*, 1998; Freidank *et al.*, 2002; Kaul *et al.*, 2000; Maraha *et al.*, 2001; Sessa *et al.*, 2001; Smieja *et al.*, 2001; Wong *et al.*, 1999).

## 2.2 Diagnosis of acute *C. pneumoniae* infection

### 2.2.1 Culture

Having earlier been considered a golden standard in the diagnosis of *C. trachomatis*, chlamydial culture has not displayed such a strong position in the case of *C. pneumoniae* (Saikku, 1994). *C. pneumoniae* culture is highly specific and most successful in HL (Cles *et al.*, 1990) and Hep-2 (Roblin *et al.*, 1992) cell lines, reaching 50 % sensitivity in the samples taken from antibody-positive subjects in acute infections (Ekman *et al.*, 1993b; Grayston *et al.*, 1993). Lowered culture sensitivity in the case of *C. pneumoniae* may be due to the relatively low amount of EBs present in swab specimen or the poor ability of EBs to form inclusion (Saikku, 1994).

### 2.2.2 Antigen detection

Commercially available enzymeimmunoassay (EIA) kits for the detection of *C. trachomatis* LPS are basically applicable to the diagnosis of *C. pneumoniae*, too. However, based on the detection of a chlamydial group antigen from respiratory tract sample, these methods do not provide species-specificity. C. *pneumoniae*, *C. psittaci* and occasionally even *C. trachomatis* can be present in the respiratory sam-

ples. Further, sensitivity is questioned by the small quantity of LPS proposed to be present in the samples (Saikku, 1994).

Another method for antigen detection is direct staining with a fluorescently labeled antibody (FA). Unlike *C. trachomatis*, *C. pneumoniae* EBs are more fragile and unstable under the harsh conditions that EBs undergo during fixation (Wang *et al.*, 1991). It is also difficult to obtain a representative sample from the respiratory tract. Overall, staining with FA yields low sensitivity (20-60 %) and a skilled reader is required to gain specificity (Saikku, 1994).

### 2.2.3 Serology

The earliest immune response in acute chlamydial infections is the development of IgM antibodies against cLPS (Ekman *et al.*, 1993b). In primary infections, IgM and IgG antibodies, measured by microimmunofluorescence (MIF), develop generally about 3 and 6-8 weeks after the first symptoms, respectively (Kuo *et al.*, 1995a). The diagnostic criteria for primary *C. pneumoniae* require 4-fold rise in the IgG titer (paired sera) and/or single titer of ≥16 for IgM (Dowell *et al.*, 2001). A weak IgM response can sometimes be observed in reinfections, when IgG and IgA antibodies appear fast, 1 to 2 weeks after the onset of illness (Grayston *et al.*, 1990). Because antibodies appear relatively late, serology has limitations in cases in which only acute phase serum is available. Further, the antibody response may be missed if the convalescent serum has been taken too early, i.e. less than 3 weeks after the onset of illness.

Complement fixation (CF) was the first method applied to chlamydial serology. Genus-specific glycolipid (cLPS) is used as a CF-antigen and thus, the method does not permit discrimination between chlamydial species. In addition, the lack of an IgM antibody response against LPS reduces the sensitivity of CF in the elderly, who usually have reinfections (Grayston *et al.*, 1990).

MIF is considered the golden standard in chlamydial diagnosis. It was first applied to the diagnosis of *C. trachomatis* infections (Wang *et al.*, 1970). According to the current recommendations for *C. pneumoniae* serology, MIF is considered the only satisfactory serological test (Dowell *et al.*, 2001). Methodologically, antibodies present in a serum sample first attach themselves to the EB antigen bound on a glass slide, and are then visualized via the binding of a secondary, FITC-labeled antibody. The method is highly specific and suitable for the measurement of IgA, IgG and IgM antibodies. The restrictive point is that the reliable reading of an MIF test results requires an experienced microscopist. Most of the MIF assays are in-house methods, but commercial assays are available, too. However, there is no standardized MIF test and different assay conditions have been shown to account for dis-

crepant results between laboratories (Peeling *et al.*, 1998). Moreover, differences between the MIF antigen used and the *C. pneumoniae* isolate to be studied may result in variations between the results (Kutlin *et al.*, 1998).

As with MIF, EIA is also able to distinguish between IgA, IgG and IgM antibody classes. In EIA, antibodies are trapped by a *C. pneumoniae* antigen bound to the solid phase (microtiter plate). The antigen-antibody reaction is detected by the enzyme-labeled secondary antibody followed by a substrate reaction. In commercial EIA kits, specificity, cut-off values, as well as the criteria for positivity vary from kit to another and make between-laboratory comparisons difficult (Boman *et al.*, 2002).

## 2.3 Diagnosis of chronic *C. pneumoniae* infection in cardiovascular diseases

Since chronic *C. pneumoniae* infection has been associated with a number of various diseases, the need for a satisfactory diagnostic method has emerged. Although viable *C. pneumoniae* has recently been successfully isolated from atherosclerotic lesions, insensitivity and difficulties in isolation prevent the use of culture in the diagnosis of chronic *C. pneumoniae*. Furthermore, in chronic conditions, *C. pneumoniae* can be penetrated into deeper sites and a representative sample may be challenging to obtain for culture or antigen detection (Saikku, 1994). At present, there are no specific and sensitive methods for the diagnosis of chronic *C. pneumoniae* infection.

### 2.3.1 Serology

Both MIF and EIA methods are used for antibody measurement in chronic *C. pneumonia*e infections. The first publication on the serological association of *C. pneumoniae* with CHD was based on the measurement of *C. pneumoniae* IgG and IgA antibodies by MIF (Saikku *et al.*, 1988). Although the presence of continuously elevated *C. pneumoniae* antibodies poorly reflects the actual site or phase of infection, it still may indicate chronic infection. The biological half-life for IgA is about one week and over three weeks for IgG. However, circulatory IgG antibodies have been suggested to persist after an acute *C. pneumoniae* infection for years (Kuo *et al.*, 1995b; Paldanius *et al.*, 2005). Moreover, the usefulness of serology is poor in assessing the role of *C. pneumoniae* in cardiovascular disease patients, since the age-matched controls are also at the age when seroprevalence can reach up to 80 % (Kalayoglu *et al.*, 2002).

The presence of elevated serum *C. pneumoniae* IgA and IgG antibodies with concomitantly increased levels of highly sensitive C-reactive protein (hsCRP) has

been associated with coronary risk, acute coronary syndromes and asthma (Huittinen *et al.*, 2003; Miya *et al.*, 2004; Sävykoski *et al.*, 2004). Moreover, the presence of persistently elevated *C. pneumoniae* IgA titer together with elevated hsCRP levels is a significant risk factor combination for coronary events (Huittinen *et al.*, 2003).

### 2.3.2 Immune complexes

During *C. pneumoniae* infection, immune complexes containing cLPS (Leinonen *et al.*, 1990) or chlamydial proteins (Linnanmäki *et al.*, 1993) can be present in circulation. Continuous production of chlamydial antigens, such as cLPS, during chronic infection may lead to the continuous presence of circulating immune complexes in serum. Indeed, elevated levels of cLPS-IgM immune complexes have been demonstrated in coronary heart disease patients as a sign of the presence of *C. pneumoniae* in the blood stream (Leinonen *et al.*, 1990; Saikku *et al.*, 1992).

### 2.3.3 Polymerase chain reaction (PCR)

Although PCR enables the detection of even nonviable *C. pneumoniae*, the goal to use PCR as a reliable diagnostic method is still far from resolved. Boman et al. were the first to detect the *C. pneumoniae* Omp1 gene by nested PCR in the PBMCs of cardiac patients (Boman *et al.*, 1998). However, troubles both in the specificity and sensitivity as well as in the validation of new assays exist. In addition, PCR correlates poorly with MIF serology. Differences in DNA extraction methods and PCR protocols have been suggested to account for variations in results. Indeed, samples from same patients have produced significantly different PCR results when investigated in different laboratories (Apfalter *et al.*, 2001; Ramirez, 1996).

### 2.3.4 Other methods

In addition to PCR, *C. pneumoniae* has been detected from vascular tissue by several other methods, including immunocytochemistry (ICC) and electron microscopy (EM). The agreement among these methods is, however, very poor, with a low percentage of samples detected positive with the two methods (Campbell *et al.*, 1995; Grayston *et al.*, 1995; Kuo *et al.*, 1993a; Kuo *et al.*, 1995a; Kuo *et al.*, 1993b; Ong *et al.*, 1996). *C. pneumoniae* has also been detected in human atherosclerotic plaques and aortic valves by i*n situ* hybridization, which allows for the localization and quantification of chlamydial DNA (Mosorin *et al.*, 2000; Pierri *et al.*, 2005).

## 2.4 *C. pneumoniae* mouse models for atherosclerosis

Animal models are useful tools in assessing the causality of pathogens and their impact on various diseases, e.g. on cardiovascular diseases. Both rabbits and mice are generally used in elucidating the course of *C. pneumoniae* infection and its role in the initiation and progression of atherosclerosis.

### 2.4.1 *C. pneumoniae* infection

Intranasal inoculation with *C. pneumoniae* causes self-limited pneumonia in mice (Kaukoranta-Tolvanen *et al.*, 1993; Yang *et al.*, 1993). IgG antibodies against *C. pneumoniae* begin to rise 10 to 12 days after the infection and the highest titers can be observed 3-4 weeks post infection, whereas maximum IgM levels are seen in 2-3 weeks (Kaukoranta-Tolvanen *et al.*, 1993; Yang *et al.*, 1993).

The clearance rate seems to be dependent on the *C. pneumoniae* isolate, the dose administered as well as on the mouse strain used (Erkkilä *et al.*, 2000; Kaukoranta-Tolvanen *et al.*, 1993; Yang *et al.*, 1993). When administred with low doses of *C. pneumoniae*, inflammatory changes in the lung occur more slowly, but can be detected even after several weeks post inoculation (Kaukoranta-Tolvanen *et al.*, 1993). Viable *C. pneumoniae* can be isolated generally up to 4 weeks, but sometimes even up to 6 weeks after infection (Kaukoranta-Tolvanen *et al.*, 1993; Penttilä *et al.*, 1998; Penttilä *et al.*, 1999; Yang *et al.*, 1993). After multiple inoculations, histopathological changes in the lung may appear for up to 4 months (Moazed *et al.*, 1997).

After recovery, latent *C. pneumoniae* may remain in the lungs. Latent forms can be reactivated and isolated from lung tissue after immunosuppressive cortisone therapy (Laitinen *et al.*, 1996; Malinverni *et al.*, 1995).

### 2.4.2 *C. pneumoniae*, serum lipids and atherosclerosis

Mice as well as other rodents, like rats, are very resistant to atherosclerosis. Among normal mouse strains, the only atherosclerosis-prone inbred strain is C57BL/6. In these mice, atherosclerotic lesions develop under a hyperlipidemic diet (Blessing *et al.*, 2001) or even under a small cholesterol supplement in the diet (Erkkilä *et al.*, 2004). However, the lesions differ in their pathology and location from human lesions. To create conditions in which lesions resembling those in human atherosclerosis develop, genetically modified animals and/or a hyperlipidemic diet are needed. For this purpose, animals with a deficiency in lipoprotein metabolism like the LDL receptor knockout (LDLR KO) mice (Ishibashi *et al.*, 1993) and apoE knockout (apoE KO) mice (Plump *et al.*, 1992) have been used.

Several studies with mice have revealed that intranasally inoculated *C. pneumoniae* can disseminate from the lungs to the aorta and other tissues and, further, that *C. pneumoniae* promotes and accelerates atherosclerosis in mice. Various mouse strains differ in their susceptibility to *C. pneumoniae* infection and atherosclerosis (Paigen *et al.*, 1990). Despite the postulation that *C. pneumoniae* is not an independent risk factor for atherosclerosis, but rather acts in concert with high serum lipids (Blessing *et al.*, 2001; Blessing *et al.*, 2002; Ezzahiri *et al.*, 2002; Hu *et al.*, 1999; Liu *et al.*, 2000; Moazed *et al.*, 1999), there are some studies in normolipidemic mice emphasizing the role of *C. pneumoniae* in triggering inflammation in the aorta and the progression of atherosclerosis (Blessing *et al.*, 2000; Erkkilä *et al.*, 2004). Baseline cholesterol levels among different mouse strains vary strongly and are dependent on genetic background (Table 2.).

***Table 2. Serum total cholesterol levels among mouse strains.***

| Mouse strain | Serum total cholesterol (mmol/l) | |
|---|---|---|
| | Regular chow | High fat diet |
| NIH/S | 2.5-3.5 | 5-6 |
| C57Bl/6J | 2.5-3.5 | 5-6 |
| •LDLR KO | 5-10 | 35-40 |
| •ApoE KO | 10-15 | 35-40 |
| ApoE3-Leiden | 10-15 | 35-40 |

C57BL/6J mouse model

The susceptibility of C57BL/6J mice to atherosclerosis has been shown to be due to the s-allele form in the Ath-1 gene in chromosome 1 (Paigen *et al.*, 1987). During a normal chow diet in C57BL/6J mice cholesterol levels, mainly in HDL, are about 2.5-3.5 mmol/l and during a high fat diet can rise up to 3-fold with the majority of cholesterol being distributed in the LDL and VLDL fractions (Paigen *et al.*, 1987). No lesion formation is detected under normal chow, whereas first stage lesions with macrophage-foam cells are formed in a subendothelial intimal space near the aortic valve during a high fat diet (Paigen *et al.*, 1985).

In C57BL/6J mice, persistent infection in the aorta can be established by multiple *C. pneumoniae* inoculations (Campbell *et al.*, 1998). *C. pneumoniae* infection alone in normolipidemic C57BL/6J mice causes inflammatory changes and intimal

thickening in the aorta (Blessing *et al.*, 2000), which are characterized as very early stages in the atherosclerotic process (Stary *et al.*, 1995), although no lesions can be detected (Blessing *et al.*, 2000). Erkkilä et al. recently demonstrated after two *C. pneumoniae* reinfections that C57BL/6J mice on a 0.2 % cholesterol supplement diet develop larger early lesions in the aorta compared to uninfected control mice (Erkkilä *et al.*, 2004). Erkkilä et al. did not see any changes in serum total lipids, nor did Blessing et al., who reported that *C. pneumoniae* in combination with a hyperlipidemic diet enhances aortic lesion formation in C57BL/6J mice (Blessing *et al.*, 2001). This accelerating effect of *C. pneumoniae* on lesion formation in C57BL/6J mice has been demonstrated to be partly mediated via the induction of TNF-α production, since the effect of *C. pneumoniae* can be abolished in the p55, TNF-α receptor, KO mice (Campbell *et al.*, 2005). Another additional mechanism behind the atherogenic effect of *C. pneumoniae* may be the due to increased inducible nitric oxide synthase (iNOS) activity (Chesebro *et al.*, 2003). iNOS is expressed in a variety of cell types as a response to inflammation and cytokines (Kleinert *et al.*, 2003).

Details in the study design and execution are critical in *C. pneumoniae* mouse models. Differences in the experimental conditions, such as the *C. pneumoniae* dose and infection intervals may account for the negative results with C57BL/6J mice (Caligiuri *et al.*, 2001).

### ApoE KO and ApoE3-Leiden mouse models

ApoE is a glycoprotein essential for the clearance of VLDL and chylomicron remnants (Meir *et al.*, 2004). As a consequence of apoE deficiency, serum total cholesterol in homozygous apoE KO mice is elevated up to 10-15, primarily in the VLDL fraction. Moreover, triglyceride levels are approximately 70 % higher than those in normal mice (Plump *et al.*, 1992). ApoE KO mice spontaneously develop atherosclerosis even under regular chow (Nakashima *et al.*, 1994). Lesion formation can be accelerated and exacerbated with a high fat diet, while VLDL cholesterol levels can be further elevated about 3-fold (Plump *et al.*, 1992). Lesions observed in the apoE KO mice resemble those detected in humans, i.e. mice develop advanced lesions with fibrous caps at vascular branch points (Reddick *et al.*, 1994). Primary *C. pneumoniae* infection in hyperlipidemic apoE KO mice causes an elevation in triglycerides, although no effect on lesion formation was observed (Aalto-Setälä *et al.*, 2001). Secondary *C. pneumoniae* infection, however, even in normolipidemic apo E KO mice, increases lesion size in the aorta and induces a significant increase in serum total triglyceride levels (Rothstein *et al.*, 2001). Several differences in the study design, such as *C. pneumoniae* strain and dose, infection intervals as well as the site of lesions to be analyzed may account for the conclusion of the minor role of

*C. pneumoniae* as a risk factor in the atherosclerosis in apoE KO mice (Aalto-Setälä *et al.*, 2001; Caligiuri *et al.*, 2001).

Another mouse model, transgenic ApoE3-Leiden mice on a C57BL1/6J background, was used when studying the effects of *C. pneumoniae* infection on lesion progression (Ezzahiri *et al.*, 2002). The ApoE3-Leiden mutation in the apoE gene is associated with the predominantly inherited form of familial type III hyperlipoproteinemia (Havekes *et al.*, 1986). ApoE3-Leiden transgenic mice display primarily in elevated levels of VLDL and LDL. In ApoE3-Leiden mice, *C. pneumoniae* promotes the development of more complex lesions, which shift towards more severe and complex lesions with fibroatheroma in nine months (Ezzahiri *et al.*, 2002).

#### LDLR KO mouse model

LDLR KO mice were originally created for a model of familial hypercholesterolemia (FH). The serum lipid abnormalities in LDLR KO mice are not so serious compared to those observed in apoE KO mice. The majority of the serum total cholesterol is found in the LDL fraction. During chow, serum total cholesterol is about 5-10, and mice do not develop atherosclerosis unless fed with a Western type of high fat diet, in which cholesterol levels rise up to 6-fold and in the intermediate fibro-proliferative stage, large lesions are formed (Ishibashi *et al.*, 1994). Serum lipids and lipoproteins remain unchanged during multiple *C. pneumoniae* infections (Hu *et al.*, 1999; Liu *et al.*, 2000). However, *C. pneumoniae* infection increases lesion size and intima thickening and generates more severe lesions with necrotic cores and fibrous caps in LDLR KO mice in nine months (Hu *et al.*, 1999). The atherogenic effect of *Chlamydiae* has been demonstrated to be specific for *C. pneumoniae*, and dependent on simultaneous cholesterol supplement in the diet (Hu *et al.*, 1999).

## 2.5 LPS metabolism

In gram-negative bacterial infections, LPS is one of the most potent inducers of an inflammatory reaction by activating a wide variety of cell types. A small amount of LPS is beneficial to mount an immune defence, whilst exposure to large amounts of LPS may trigger massive destructive cellular responses. Therefore, a host's mechanisms for endotoxin inactivation are essential.

The detoxification process includes the incorporation of the lipid A part into the phospholipid-rich surface of lipoproteins (Read *et al.*, 1993). The major lipoprotein classes, HDL, LDL, VLDL and chylomicrons (CMs) have been demonstrated to be able to sequester and neutralize LPS (Harris *et al.*, 1990; Harris *et al.*, 1993; Netea *et al.*, 1998; Ulevitch *et al.*, 1979; Van Lenten *et al.*, 1986; Victorov *et al.*, 1989).

During clearance, the endotoxin anchored to lipoproteins is shunted to hepatocytes via receptor-mediated uptake and further excreted in bile (Read *et al.*, 1993).

The lipopolysaccharide binding protein (LBP) is a 60 kDa acute phase protein (Schumann *et al.*, 1996; Schumann *et al.*, 1999) which is elevated during bacterial infections. LBP belongs to the LPS binding/lipid transfer protein family together with phospholipid transfer protein (PLTP), cholesterol ester transfer protein (CETP) and neutrophil bacteridical permeability increasing protein (BPI). Although principally produced by the liver, LBP is also expressed in lungs, kidneys and heart (Schumann *et al.*, 1996; Schumann *et al.*, 1999). In the liver, LBP expression can be induced by IL-1 and IL-6 (Grube *et al.*, 1994; Ramadori *et al.*, 1990; Wan *et al.*, 1995). In normal human serum, the LBP concentration ranges from 5-10 μg/ml increasing up to 10-30 fold during the acute phase response (APR) (Blairon *et al.*, 2003; Froon *et al.*, 1995; Opal *et al.*, 1999).

LBP has a pivotal role in LPS metabolism. In circulation, LBP is able to facilitate the neutralization of LPS by transferring LPS from the bacterial outer membrane or LPS aggregates to HDL (Hailman *et al.*, 1996a; Van Lenten *et al.*, 1986; Vesy *et al.*, 2000). HDL-associated LPS can be subsequently delivered to LDL or VLDL (Levels *et al.*, 2001), a process accelerated in the presence of LBP and PLTP (Levels *et al.*, 2005). PLTP and LBP are boomerang-shaped proteins containing two lipid binding pockets apparently important in the transfer of LPS (Huuskonen *et al.*, 1999). LBP was first found in close association with free apoA-I and apoA-I-containing HDL particles (Massamiri *et al.*, 1997; Wurfel *et al.*, 1994). Contradictory data has also been reported suggesting that circulatory LBP is associated mainly with apoB-containing lipoproteins (LDL and VLDL) *in vivo* both in healthy individuals and in patients with an inflammatory condition (Vreugdenhil *et al.*, 2001).

Another pathway in LPS metabolism is capable of triggering an inflammatory response. LBP may result in target cell activation and lead to the release of inflammatory mediators and cytokines by transferring LPS to either membrane bound (mCD14) or soluble CD14 (sCD14) protein. The mCD14 is a 55 kDa, LPS responsive pattern-recognition receptor protein on the surface of monocytes and neutrophils (Viriyakosol *et al.*, 1996). Endothelial and human coronary artery smooth muscle cells (HCASMCs), previously considered to be CD14 negative cells, have recently been shown to express functional mCD14 (Jersmann *et al.*, 2001; Stoll *et al.*, 2004). Serum sCD14 is a prerequisite for the activation of CD14 negative cells by LPS but has also been shown to participate in the activation of CD14 expressing cells (Frey *et al.*, 1992; Hailman *et al.*, 1994; Hailman *et al.*, 1996b; Kitchens *et al.*, 1998).

The mCD14 has neither a transmembrane nor cytoplasmic domain and thus, accessory proteins are required to mediate intracellular signal transduction. One of

these accessory proteins is a Toll-like receptor 4 (TLR-4), a member of the Toll-like receptor family (Means *et al.*, 2000), which has been demonstrated to be connected to the endotoxin-CD14 complex (Chow *et al.*, 1999). Another substantial LPS recognition protein of the receptor complex is MD-2, which has been shown to bind both LPS and CD14 (Gioannini *et al.*, 2004; Nagai *et al.*, 2002; Shimazu *et al.*, 1999; Viriyakosol *et al.*, 2001). Eventually, after multiple interactions with intracellular proteins, cellular activation by LPS leads to the activation of the nuclear factor κB (NF-κB) and its translocation to the nucleus followed by the expression of inflammatory cytokines (Fitzgerald *et al.*, 2004).

The distribution of LPS in serum is strongly dependent on the concentrations of LPS-binding/associated proteins. For example, LBP-mediated LPS transfer to lipoproteins has been shown to be enhanced in the presence of sCD14 (Wurfel *et al.*, 1995). Also, the LBP/sCD14 ratio may determine the targeting of LPS transfer in circulation. During APR, sCD14 levels increase maximally by 2-fold whereas LBP levels are elevated by 10-30-fold (Froon *et al.*, 1995). An increased LBP/sCD14 ratio is appropriate for enhanced LPS clearance rather than cellular activation (Landmann *et al.*, 1995). Accordingly, the lower LBP/sCD14 observed in chronic infections may implicate enhanced capacity of LPS to activate cells (Tapping *et al.*, 2000).

## 2.6 Infection, inflammation and lipoprotein metabolism

The body's response to infection and inflammation is known as the acute phase response (APR), which is responsible for the release of several cytokines. Cytokines mediate multiple changes in lipid metabolism. The direction of change in cytokine pools and thereby in lipid levels during human infections varies between pathogens. Further, the direction and extent of inflammatory alterations in the distribution of lipids are species-specific (Feingold *et al.*, 1993a; Grunfeld *et al.*, 1992; Sammalkorpi *et al.*, 1988).

### 2.6.1 Overview on lipoprotein metabolism

Lipoprotein metabolism can be divided into two major pathways: the intestine-derived exogenous and the liver-derived endogenous pathway. Dietary lipids, cholesterol and triglycerides, are delivered by the exogenous pathway and absorbed via intestinal enterocytes where they are assembled into chylomicrons (CMs). Once entered into general circulation, lipoprotein lipase (LPL) hydrolyses the triglyceride-rich core of CM-producing chylomicron remnants. Chylomicron remnants are taken up by the liver preferentially via the LDL-receptor related protein (LRP). In addition, LDL-receptor (LDLr) can also be involved in this process (Packard *et al.*, 1999).

In the endogenous pathway, lipids (triglycerides, cholesterol, phospholipids) in the liver are assembled and excreted in the form of VLDL. The main apolipoprotein in VLDL is apolipoprotein B 100 (apoB-100). LPL hydrolyzes the triglycerides in the VLDL particle, resulting in the formation of phospholipid-rich surface components and VLDL remnants. Via LPL action, VLDL remnants can be further transformed to intermediate density lipoprotein (IDL). Phospholipid surface remnants are transferred to HDL by phospholipid transfer protein (PLTP) (Jiang *et al.*, 1999), a process important in the maintenance of serum HDL levels. IDL particles are further hydrolyzed by LPL and hepatic lipase (HL), resulting in the formation of LDL (Goldberg, 1996; Nilsson-Ehle *et al.*, 1980). Cholesterol-rich LDL particles are cleared from circulation mainly via LDL receptors (Brown *et al.*, 1986). Antiatherogenic HDL is biosynthesized with apoA-I as the major apolipoprotein component in the liver, but some amount of the apoA-I also originates from the intestine (Barter *et al.*, 2003; Eisenberg, 1984; Linsel-Nitschke *et al.*, 2005).

### 2.6.2 Changes in VLDL metabolism during inflammation

One of the main characteristics and consistent changes in lipid levels during APR is the elevation of serum triglycerides (Alvarez *et al.*, 1986; Cox *et al.*, 1975; Fiser *et al.*, 1972; Friedland *et al.*, 1982; Vergani *et al.*, 1978). In experimental animals, low doses of LPS, used to mimic mild infection, causes the production of fatty acids by increased adipose tissue lipolysis and hepatic *de novo* fatty acid synthesis as well as the decreased hepatic fatty acid β-oxidation. Fatty acids are then esterified into triglycerides and assembled into VLDL particles in the liver (Memon *et al.*, 1992; Memon *et al.*, 1993). In contrast, administration with higher LPS doses reduces the activity of LPL and the expression of apoE, both of which are essential for proper VLDL clearance (Feingold *et al.*, 1992; Hardardottir *et al.*, 1997; Tripp *et al.*, 1993). In humans, serum total and VLDL triglycerides are elevated during inflammatory conditions (Alvarez *et al.*, 1986; Grunfeld *et al.*, 1991; Khovidhunkit *et al.*, 2004; Sammalkorpi *et al.*, 1988).

### 2.6.3 Changes in LDL and HDL metabolism during inflammation

Another inflammatory change in human lipids during infection is the decrease in serum total cholesterol (Sammalkorpi *et al.*, 1988; van Leeuwen *et al.*, 2003). The mechanisms behind the decrease in cholesterol are not entirely clear. In animals, cholesterol changes are species-specific and dependent on infective agent as well as the severity and stage of illness (Alvarez *et al.*, 1986; Feingold *et al.*, 1992; Fiser *et al.*, 1972; Fiser *et al.*, 1971; Gallin *et al.*, 1970; Ziegler *et al.*, 1992). Although LPS and some cytokines, such as TNFα and IL-1, activate in rodents 3-hydroxy-3-

methylglutaryl coenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in cholesterol biosynthesis pathway, only a moderate increase is observed in hepatic or serum cholesterol levels (Feingold *et al.*, 1993a; Feingold *et al.*, 1995; Hardardottir *et al.*, 1994). The explanation for the small increase in cholesterol levels by LPS and cytokines is the decreased activity of squalene synthase, another enzyme downstream in the cholesterol synthesis pathway (Memon *et al.*, 1997).

On the basis of human studies, total cholesterol reduction seems to be related to the severity of infection rather than to the causative agent (Sammalkorpi *et al.*, 1988; van Leeuwen *et al.*, 2003), although a correlation to either ones is not always demonstrated (Alvarez *et al.*, 1986). The decline in cholesterol can be ascribed to the reduction of esterified cholesterol mainly in LDL and but also in the HDL fraction (Gordon *et al.*, 1996; Kitchens *et al.*, 2003; Sammalkorpi *et al.*, 1988; van Leeuwen *et al.*, 2003). *In vitro* studies using human HepG2 cells indicate that IL-1 and IL-6 decrease apolipoprotein and cholesterol accumulation in a medium by affecting cholesterol synthesis and secretion or both (Ettinger *et al.*, 1994).

Infection and inflammation do not merely affect HDL lipid levels but also change the levels of several HDL-associated proteins (Fig. 4). Concentrations of apoA-I and paraoxonase (PON) are reduced (Clifton *et al.*, 1985; Van Lenten *et al.*, 2001), whereas the level of HDL-associated acute phase protein, serum amyloid A (SAA), can be elevated up to 1000-fold (Cabana *et al.*, 1989; Kisilevsky *et al.*, 2002; Van Lenten *et al.*, 1995). SAA displaces apoA-I (Cabana *et al.*, 1999; Coetzee *et al.*, 1986) and PON (Cabana *et al.*, 2003) from the surface of HDL particles. PON has an important antioxidant function by metabolizing lipid peroxides and protecting against their accumulation in LDL (Aviram *et al.*, 2000; Draganov *et al.*, 2000) and, consequently, decreased PON activity during the acute phase may contribute to the decreased antioxidant potential of HDL (Van Lenten *et al.*, 1995). Another enzyme protecting LDL from oxidation is platelet-activating factor-acetylhydrolase (PAF-AH), of which about 30 % is bound in HDL in humans (Stafforini et al., 1997). The effects of APR on PAF-AH are controversial, varying among animals (Howard et al., 1997; Van Lenten *et al*., 1995). In humans, PAF-AH activity increases significantly in several diseases (Claus *et al.*, 2005; Endo *et al*., 1994; Khovidhunkit *et al*., 1999). The anti-inflammatory phenotype of HDL is changed to pro-inflammatory type due to these protein changes.

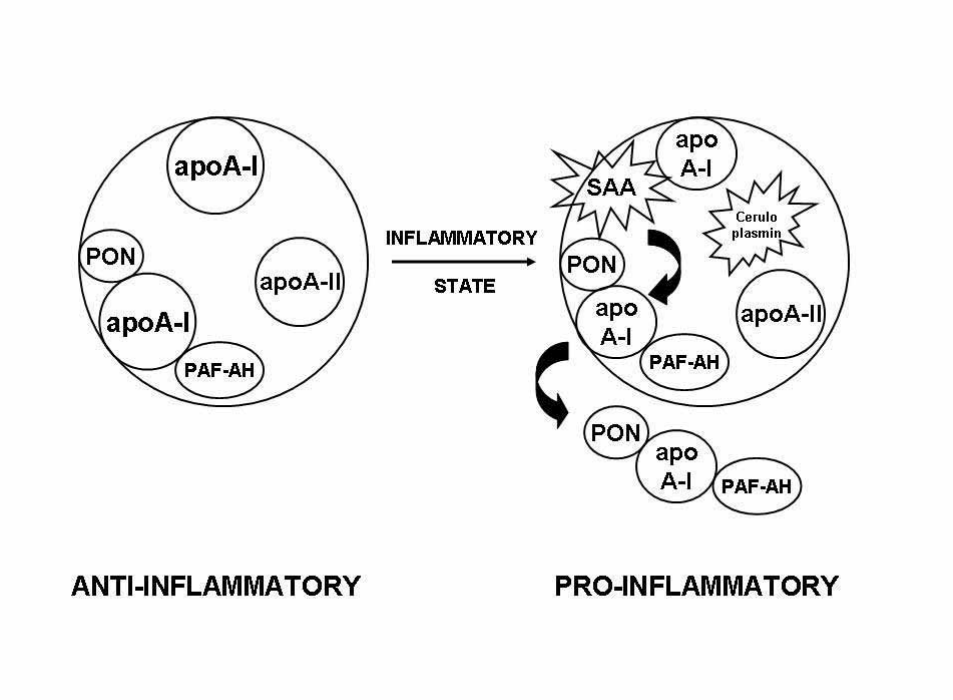


***Fig. 4. Proinflammatory properties in HDL generated during inflammation. SAA, serum amyloid A; PON, paraoxonase; PAF-AH, platelet-activating factor-acetylhydrolase.***

During the acute phase, alterations in some key regulatory enzymes involved in HDL metabolism are observed, including decreased lecithin-cholesterol acyltransferase (LCAT), cholesterol ester transfer protein (CETP) and HL activities (Feingold *et al.*, 1999; Hardardottir *et al.*, 1996; Khovidhunkit *et al.*, 2001; Ly *et al.*, 1995; Sammalkorpi *et al.*, 1988). In contrast, activity of the other serum lipid transfer protein, PLTP is increased (Barlage *et al.*, 2001).

The main regulatory pathways in HDL metabolism during inflammation are described in Figure 5. The primary function of LCAT is to convert free cholesterol into cholesterol esters in HDL particles (Barter *et al.*, 1999). Esterified cholesterol in HDL is exchanged by the function of CETP for VLDL triglycerides (Tall, 1993). Subsequently, HDL becomes depleted in cholesterol esters and triglyceride-enriched and thus, displays good substrate properties for HL (Barrans *et al.*, 1994). HL metabolizes these large triglyceride-enriched HDL into small size HDL particles with a concomitant formation of preβ-HDL. According to current knowledge, small HDL particles can be metabolized via the kidney function (Kozyraki *et al.*, 1999). Preβ-HDL is the initial acceptor for peripheral cell cholesterol in a reverse cholesterol

transport (RCT) pathway (Fielding *et al.*, 1995). PLTP transfers phopholipid between HDL subclasses and VLDL and LDL and promotes a fusion of small size HDL particles into large size HDL with a subsequent release of preβ-HDL (Jauhiainen *et al.*, 1993; Nishida *et al.*, 1993; Tollefson *et al.*, 1988).

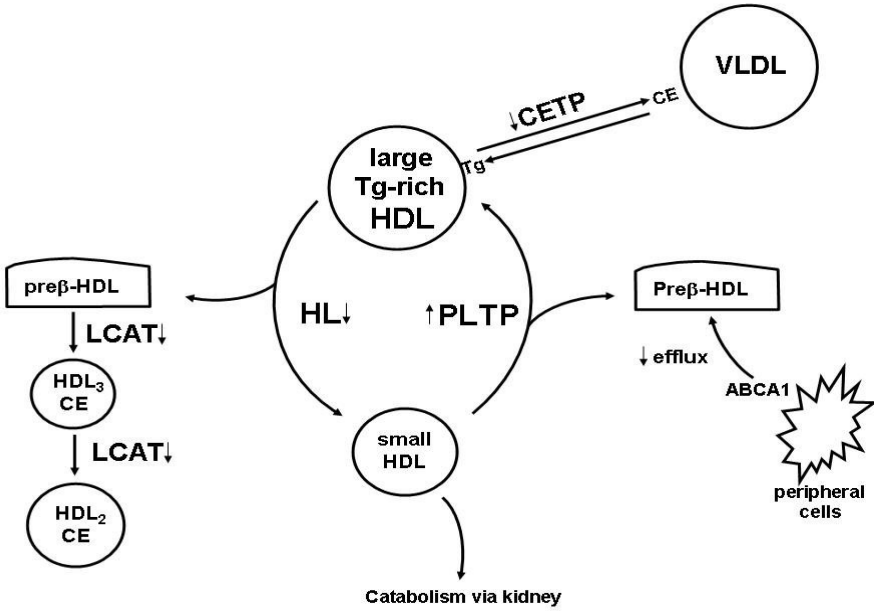


***Fig. 5. Inflammation and regulation of HDL subclasses. LCAT, lecithin-cholesterol acyltransferase; PLTP, phospholipid transfer protein; HL, hepatic lipase, CETP, cholesterol ester transfer protein; ABCA1, ATP-binding cassette transporter A1.***

Although the changes in lipid metabolism during infection are generally transient, a prolonged inflammatory state, however, can be detrimental to the host. It has been proposed that in the acute phase, HDL loses its unique properties as anti-inflammatory and antiatherogenic lipoprotein (Van Lenten *et al.*, 2001). First, the function of HDL in the reverse cholesterol transport process from the peripheral cells may be impaired. In addition, recent data also demonstrates that LPS directly downregulates the gene expression of the two HDL receptors, the ATP-binding cassette transporter A1 (ABCA1) and scavenger receptor B1 (SR-B1) involved in cholesterol efflux (Baranova *et al.*, 2002). Secondly, the ability of HDL to protect

LDL against oxidation may also be reduced (Khovidhunkit *et al.*, 2000). Thirdly, although LDL levels generally decrease during infection, the formation of highly atherogenic small dense LDL (sLDL) has been detected (Feingold *et al.*, 1993b). All these changes in lipoproteins are characterized as pro-atherogenic.

Several lines of evidence demonstrate that *C. pneumoniae* may contribute to atherosclerosis via lipoprotein metabolism. For instance, infection by *C. pneumoniae* is associated with decreased HDL cholesterol and increased triglyceride concentration (Laurila *et al.*, 1997; Murray *et al.*, 1999). Further, *C. pneumoniae* have been shown to promote LDL oxidation and macrophage foam cell formation *in vitro* (Dittrich *et al.*, 2004; Kalayoglu *et al.*, 1998b; Kalayoglu *et al.*, 1999). Finally, the direct demonstration of *C. pneumoniae* in atherosclerotic plaques (Shor *et al.*, 1992) demonstrates its ability to disseminate from the respiratory tract to circulation and particularly, to the site of lesion formation.

## 2.7 Statins as cardiovascular drugs

Statins are HMG-CoA reductase inhibitors. Statins prevent hepatic cholesterol biosynthesis by blocking the HMG-CoA reductase, an enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis (Endo *et al.*, 1977). Although statins are widely used in the treatment of hypercholesterolemia, several lines of evidence indicate that statins do not only decrease endogenous cholesterol synthesis but they possess anti-inflammatory effects. Overall, statin treatment has reduced morbidity and mortality in CHD patients (Pedersen, 1994; Pedersen *et al.*, 1998; Shepherd *et al.*, 1995).

### 2.7.1 Lipid lowering effect

In humans, statins cause a reduction in serum LDL cholesterol and an elevation in HDL cholesterol. Various statins differ in their ability to lower LDL cholesterol levels in serum (Vaughan *et al.*, 2004). A reduction in LDL levels is caused by both enhanced LDL uptake and decreased LDL secretion. Decreased liver cholesterol synthesis leads to the activation of sterol regulatory element-binding proteins (SREBP) and enhances the expression of their target genes, including LDL receptors (Brown *et al.*, 1997). Subsequently, the clearance of LDL and LDL precursors from the circulation is increased. Statins have also been suggested to inhibit the apoB-100 synthesis in the liver, leading to a reduction in the synthesis and secretion of triglyceride-rich lipoproteins, VLDL (Vega *et al.*, 2003). During statin medication, VLDL levels are further decreased due to decreased serum triglycerides (Bakker-Arkema *et al.*, 1996; Stein *et al.*, 1998).

The mechanism by which statins increase HDL is not entirely known. In fact, the increase in HDL cholesterol achieved by statin use is relatively low (5-10 %). Despite the ability of statins to induce apoA-I production, a large variation in apoA-I induction capacity has been observed (Maejima *et al.*, 2004).

### 2.7.2 Anti-inflammatory effect

Cholesterol lowering is not the only beneficial effect yielded by statins. Statins have been shown to decrease serum CRP levels, improve endothelial function, stabilize atherosclerotic plaques and possess anti-inflammatory effects (Albert *et al.*, 2001; Jialal *et al.*, 2001; Kinlay *et al.*, 2003; Ridker, 2003; Ridker *et al.*, 2001).

#### Anti-chlamydial effect

The ameliorating effect of simvastatin during *C. pneumoniae* infection has been reported (Erkkilä *et al.*, 2005). The mechanism by which the anti-inflammatory effect of statins is mediated is not completely understood. *C. pneumoniae*-infected macrophages and endothelial cells release a decreased amount of MCP-1 and IL-8 when coincubated with cerivastatin. Supporting this finding, cerivastatin decreases the activation of NF-κB, a protein which regulates the expression of several cytokines and chemokines, including IL-8 and MCP-1. The inactivation of NF-κB is mediated via the Rho signaling pathway, and inhibition of this pathway leads to decreased NF-κB activity in *C. pneumoniae* infected cells (Dechend *et al.*, 2003).

# 3 AIMS OF THE STUDY

Different animal models have been utilized to elucidate the role of *C. pneumoniae* in the development of atherosclerosis. Although *C. pneumoniae* is linked to pro-atherogenic serum lipid profiles, little is known about its effects in mice. Moreover, the beneficial effects of statins on lipid profile and inflammation have been reported, but whether the statin therapy modulates *C. pneumoniae* infection in a mouse model is not known. Therefore, the aims in the present mouse studies were:

I: To investigate the effect of acute *C. pneumoniae* infection on serum lipids, lipoprotein distribution and lipolytic enzymes in an NIH/S mouse model.

II: To investigate the effect of pravastatin treatment on *C. pneumoniae* infection, infection-induced inflammatory response and serum total lipids in an NIH/S mouse model.

Atherosclerosis is considered to be an inflammatory disease and a link between serum highly sensitive CRP (hsCRP) and cardiac events has been demonstrated. Although several studies have suggested an association between *C. pneumoniae* infection and atherosclerosis, the lack of reliable direct methods for the diagnosis of chronic *C. pneumoniae* infection has hampered these studies and contradictory findings have been reported. Therefore, the specific aims of the human studies were:

III: To develop a specific enzyme immunoassay (EIA) method for cLPS quantification in serum.

IV: To investigate the correlation between serum hsCRP and cLPS levels in patients with acute coronary syndrome (ACS) during a one-year follow-up period.

# 4 MATERIALS AND METHODS

## 4.1 Materials

### 4.1.1 Experimental animals (I, II)

Female NIH/S (National Institute of Health/S) mice were purchased from the National Public Health Institute (Animal Care Center, Kuopio, Finland). The mice were fed either an autoclaved regular diet (Altromin, Chr. Petersen A/S, Ringsted, Denmark) or an high-fat diet (21 % total fat, 0.2 % cholesterol and 19.5 % casein, Harlan Teklad, NM Horst, the Netherlands).

### 4.1.2 Human subjects (III, IV)

Acute coronary syndrome (ACS) patients were recruited between September 1998 and December 2000 from nine Finnish hospitals. The ethics committees of the participating hospitals approved the study, and all patients gave written informed consent. Serum samples were collected from patients with ACS within 48 hours after admittance to the hospital. Patients with the following criteria were included: subjects who entered hospital with prolonged chest pain with documented changes in ST-T waves indicating either unstable angina or a non-Q-wave myocardial infarction. During the follow-up time, samples were taken one week, three months and one year after the ACS event. Age- and sex-matched healthy blood donors obtained from Finnish Red Cross served as controls in the study III.

## 4.2 Methods

### 4.2.1 Inoculation of NIH/S mice (I, II)

Intranasal inoculation of *C. pneumoniae* was given during methoxyflurane anesthesia. The challenge dose was 5.3 $*10^5$ inclusion forming units (IFU)/mice in a saccharose-phosphate-glutamate (SPG) buffer. The control mice were inoculated similarly with (SPG). Serum samples were taken at -3, 0, 3, 6, 9, 14, and 20 days postinfection. Serum and tissue samples from 6 mice/group were collected after a fasting period of four hours three days prior to inoculation (day -3) at inoculation day (day 0), and 3, 6, 9, 14 and 20 days post *C. pneumoniae* infection (p.i.).

### 4.2.2 Statin administration (II)

Pravastatin treatment was given daily as an intraperitoneal injection in a 100 µl volume starting three days prior to infection (day -3) and continuing thereafter to the end of the follow-up period. The pravastatin dosage was 0.5 mg/kg/day. The inoculation and sampling were carried out as described (see chapter 4.2.1).

### 4.2.3 Monoclonal a-LPS antibodies

Mouse monoclonal a-LPS antibodies produced against *C. trachomatis* LPS were purchased from Medix Biochemica (Espoo, Finland). In EIA development, three monoclonal antibody clones 6701 ($IgG_1$), 6703 ($IgG_{2a}$) and 6709 ($IgG_{2a}$) were tested.

### 4.2.4 cLPS-enzyme immunoassay (III)

Microtiter plates were coated with a mouse monoclonal antibody (0.5 µg/well) specific to chlamydial LPS for 16 h at +20 °C. Blocking of non-specific binding sites was performed by phosphate-buffered saline (PBS) supplemented with 0.2 % defatted milk (1 h at +37 °C).

Serum samples were diluted 1/50 in 0.1 % octylglucoside-PBS, and LBP at 1 µg/ml in 0.1 % Tween and added together to the plate (60 µl of each). The mixture was incubated for 90 min at +37 °C. During incubation, LBP solubilizes LPS from complexes in the serum by binding to the lipid A portion of LPS. The sugar part of LPS then binds to the monoclonal a-LPS antibody coated on the plate. Polyclonal rabbit anti-LBP antibody (100 µl of 1/500 dilution in Tween-PBS), added next, binds to LBP during a one-hour incubation at +37 °C. Finally, detection is carried out by adding peroxidase-labeled goat anti-rabbit antibody (GAR-HRP, 1/10 000 dilution in 0.1 % Tween-PBS supplemented with 10 % FBS) followed by incubation with an HRP-substrate for 1 h at +20 °C in the dark. After stopping the reaction with 50 µl of 3 M $H_2SO_4$, absorbance is read at 490 nm.

### 4.2.5 Other methods

The other methods used in the original articles are summarized below.

| METHOD | ORIGINAL PUBLICATION |
|---|---|
| cholesterol concentration | I - IV |
| triglyceride concentration | I- IV |
| PLTP activity | I |
| HL activity | I |
| LCAT activity | I |
| size-exclusion chromatography | I, II |
| LBP-ELISA | I, II |
| *C. pneumoniae* culture | I, II |
| MIF | I - IV |
| KDO-analysis | III |
| phenol-water extraction | III |
| SDS-PAGE | III |
| hsCRP concentration | IV |
| statistical analysis | I - IV |
| silver staining | III |

### 4.2.6 Statistics

The Mann-Whitney U-test was used to inter-group comparisons (I, II, III). Wilcoxon's signed ranks test was performed to compare the pooled serum values (LCAT-activity) between the groups within the study period (II). A chi-squared test was used to compare the proportions of LPS-positive samples between the study groups. The diagnostic applicability of cLPS for identifying ACS patients was evaluated by using the area under the receiver operating characteristic (ROC) curve. In Study IV, proportions were compared with a chi-square test or Fisher's exact test, and quantitative data with a *t*-test or the Mann-Whitney test. The correlations were calculated with Spearman's rank correlation test. P-values of $< 0.05$ were considered statistically significant.

# 5 RESULTS AND DISCUSSION

## 5.1 Effect of acute *C. pneumoniae* on lipoprotein metabolism in NIH/S mice (I)

The *C. pneumoniae* NIH/S mouse model has been generally used to investigate the infection course and techniques (Erkkilä *et al.*, 2002; Erkkilä *et al.*, 2000; Kaukoranta-Tolvanen *et al.*, 1995). Using chow-fed NIH/S mice, the aim was to study the effect of acute *C. pneumoniae* infection on serum lipids and lipolytic enzymes to clarify whether this mouse model can be utilized to draw conclusions in a human context.

### 5.1.1 Infection and inflammation

During *C. pneumoniae* infection, none of the mice showed any clinical symptoms of illness. Successful infection was confirmed by antibody measurement and lung culture. IgG antibodies started to increase 9 days p.i. and viable *Chlamydia* could be isolated by culture from infected mice on day 3 p.i. (I, Fig. 1). All mice turned culture-negative towards the end of the follow-up. LBP, a known acute phase protein, increased in both control and infected mice, peaking on day 9 p.i. (I, Fig. 4).

LBP is principally produced in the liver, where its synthesis can be stimulated by IL-1β and IL-6 (Schumann *et al.*, 1996). Elevated LBP levels in serum have been described in septic patients (Blairon *et al.*, 2003). Mouse studies have emphasized the role of LBP in initiating the host response against the pathogen but also protecting animals against the toxic effects of LPS (Fierer *et al.*, 2002; Jack *et al.*, 1997; Knapp *et al.*, 2003). Lamping et al. have reported that acute phase concentration of LBP protects mice from the effects of LPS and gram-negative bacteria (Lamping *et al.*, 1998). In light of that study, a comparable acute phase concentration of LBP was reached on day 9 in *C. pneumoniae* infected mice in the present study.

### 5.1.2 Serum lipids

We found no permanent effect of acute *C. pneumoniae* infection on serum lipids. Small differences were observed among experimental animal groups in triglycerides with the exeption of a transient significant elevation of VLDL triglycerides (I, Fig. 3) in *C. pneumoniae* infected mice.

In animals and humans, infection and inflammation induce a broad range of alterations in serum lipids as well as in enzymes involved in lipid metabolism

(Khovidhunkit *et al.*, 2004). Both bacterial and viral infections cause hypertriglyceridemia in humans (Gallin *et al.*, 1969; Grunfeld *et al.*, 1992; Sammalkorpi *et al.*, 1988). In animal studies, LPS administration is generally used to mimick infection. Subcutaneous or intraperitoneal LPS administration leads to hypertriglyceridemia in rabbits (Hirsch *et al.*, 1964; Lequire *et al.*, 1959), other rodents (Feingold *et al.*, 1993a; Feingold *et al.*, 1992; Fiser *et al.*, 1973; Gaal *et al.*, 1984; Sakaguchi *et al.*, 1979; Scholl *et al.*, 1984) and monkeys (Fiser *et al.*, 1974). Hypertriglyceridemia is characterized by increased VLDL (Hardardottir *et al.*, 1995) due to either increased VLDL production or decreased VLDL clearance depending on the LPS dose used (Feingold *et al.*, 1992). Hypertriglyceridemia induced by LPS is detected 1-2 hours after administration, disappearing thereafter relatively rapidly. Our study suggests a similar, transient elevation in triglycerides. Since we did not further analyze the enzymes regulating triglyceride metabolism in the liver, the mechanism behind triglyceride elevation on hepatic level remained unanswerd.

In agreement with other *C. pneumoniae* mouse studies (Aalto-Setälä *et al.*, 2001; Blessing *et al.*, 2001; Erkkilä *et al.*, 2004; Memon *et al.*, 1997), *C. pneumoniae* caused no constant change in serum cholesterol levels. In rodents, serum cholesterol levels increase during LPS administration due to decreased cholesterol catabolism and increased hepatic cholesterol synthesis. LPS administration increases hepatic *de novo* cholesterol synthesis by stimulating HMG-CoA reductase (Feingold *et al.*, 1993a; Feingold *et al.*, 1995), with a concomitant decrease in the activity of squalene synthase, another enzyme downstream in the cholesterol synthesis pathway (Memon *et al.*, 1997), resulting eventually in only a small increase in hepatic cholesterol synthesis and serum total cholesterol levels.

Alterations in cholesterol as a consequence of infection differ strongly among species due to substantial differences in baseline cholesterol distribution between lipoproteins. In rodents, the majority of their cholesterol is located in the HDL fraction and the minority in LDL, whereas in humans, the LDL fraction is generally most abundant. In humans, infections generate a reduction in serum total cholesterol levels (Sammalkorpi *et al.*, 1988; van Leeuwen *et al.*, 2003). Moreover, prolonged infection is associated with unfavourable cholesterol distribution (elevated LDL and decreased HDL), which has been observed in persons with serological evidence of *C. pneumoniae* infection, too (Laurila *et al.*, 1997; Laurila *et al.*, 1998; Murray *et al.*, 1999).

### 5.1.3 PLTP, LCAT and HL activities

As could be expected in light of the mild changes in serum lipids, *C. pneumoniae* infection also resulted in minor changes in the enzyme activities regulating lipoprotein metabolism. PLTP and HL were not affected by *C. pneumoniae* (I, Fig. 5B, C),

instead, in infected mice LCAT activity diminished almost 70 % from the baseline value on day 9 (I, Fig. 5A).

There are few reports showing data on the effect of infection on serum PLTP activity in mice. In humans, PLTP activity is significantly increased in patients with a systemic inflammatory response (Barlage *et al.*, 2001). Contradictory to this, injection with a high LPS (50 μg/animal) dose causes a remarkable reduction in serum PLTP activity in C57BL/6 mice (Jiang *et al.*, 1995). Downregulation of PLTP resulted in increased HDL phospholipids and HDL surface area (Jiang *et al.*, 1995), and was believed to increase the LPS binding capacity of HDL (Ulevitch *et al.*, 1978). In our study, *C. pneumoniae* infection was probably insufficiently robust to influence PLTP activity.

Another essential enzyme involved in HDL metabolism is HL, which plays a role in both the conversion of IDL to LDL as well as in the formation of small spherical and preβ-HDL particles. Decreased HL activity, seen in humans during the acute phase response (Grunfeld *et al.*, 1991; Kwong *et al.*, 1997; Levy *et al.*, 1993; Sammalkorpi *et al.*, 1988), may therefore account for multiple alterations in plasma lipoproteins. Although endotoxin administration decreases HL activity significantly in rodents (Feingold *et al.*, 1999; Kawakami *et al.*, 1986), we could not demonstrate any effect of acute *C. pneumoniae* infection on HL. Whether repeated *C. pneumoniae* infections affect HL activity or other lipolytic enzymes has not yet been investigated.

LCAT activity was found to be substantially reduced after *C. pneumoniae* infection. The effect of *C. pneumoniae* infection on LCAT has not been reported earlier. Other studies have demonstrated reduced LCAT activity after endotoxin administration in rodents (Ly *et al.*, 1995). The drop in LCAT activity leads to a declined conversion of free cholesterol to cholesterol esters and thereby is responsible for impaired the cholesterol removal ability of acute phase HDL (Khovidhunkit *et al.*, 2001).

## 5.2 Effect of pravastatin treatment on *Chlamydia pneumoniae* infection, inflammation and serum lipids in NIH/S mice (II)

### 5.2.1 Chlamydial culture from lung tissue, serum *C. pneumoniae* IgG antibodies and pulmonary inflammation

Hydrophilic pravastatin accelerated the inflammatory response to *C. pneumonia*e infection, but was not able to decrease the infection load in the lungs of NIH/S mice (II, Fig. 1). Pravastatin treatment had no effect on the serum SAA levels of infected mice during a chow diet; SAA levels during high fat were not studied.

Statins are known to possess anti-inflammatory effects in addition to their cholesterol lowering capacity. An antiproliferative effect of lovastatin during *Salmonella enterica* serovar typhimurium infection has recently been reported (Catron *et al.*, 2004). This antimicrobial effect of statins is mediated by nonsterol mevalonate products (Catron *et al.*, 2004; Diomede *et al.*, 2001). In the present study, pravastatin treatment accelerated the pulmonary inflammatory response to *C. pneumoniae* infection without any effect on chlamydial growth in the lungs or on the antibody response to infection. However, we recently demonstrated under identical experimental conditions that lipophilic simvastatin amplifies pulmonary inflammation, but also significantly decreases chlamydial counts in lung tissue in the early phase of infection (Erkkilä *et al.*, 2005).

There is one crucial difference between simvastatin and pravastatin that might provide an explanation for the diverse antichlamydial effect between these two statins. *Chlamydiae* have been shown to contain eukaryotic lipids, including cholesterol (Ziegler *et al.*, 1992). A deficiency of cholesterol synthesis genes in the chlamydial genome (Kalman *et al.*, 1999) makes *Chlamydia* dependent on host cell lipids. Indeed, there is evidence of phospholipid trafficking from host cells to *C. trachomatis* (Hackstadt *et al.*, 1995). Lipophilic statins (e.g. simvastatin) are taken up by passive diffusion into a broad range of cells, while hydrophilic statins (e.g. pravastatin) penetrate the cell by the sodium-independent bile acid transporter present only in hepatocytes (Ziegler *et al.*, 1992). Therefore, simvastatin may perturb intracellular cholesterol metabolism, which in turn hampers *C. pneumoniae* growth in alveolar macrophages.

### 5.2.2 Serum lipids

As a consequence of high fat feeding, serum total cholesterol levels increased significantly. Size-exclusion chromatography analysis indicated that the cholesterol increment was localized majorily in HDL with some elevation in apo B-containing lipoproteins (Fig. 6).

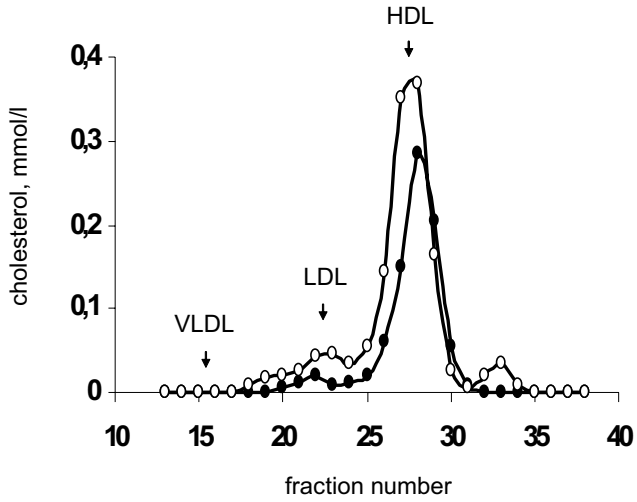


**Fig. 6.** Cholesterol distribution among NIH/S mice lipoproteins. Cholesterol distribution between lipoproteins in NIH/S control mice on regular chow (closed circle) and high fat diet (open circle) was analyzed by using a Superose 6 HR gel filtration column. The column was equilibrated with phosphate buffered saline (PBS), pH 7.4, and 200 µl of serum sample was applied to the column. The flow rate was 0.25 ml/min and 0.5 ml fractions were collected and used for cholesterol measurement.

Serum triglycerides but not cholesterol were increased by the pravastatin treatment throughout the follow-up period during high fat (II, Table 1.). Similarly, triglycerides were consistently higher by pravastatin in *C. pneumoniae* infected mice during high fat.

The triglyceride-increasing effect by pravastatin is a contradictory observation since in humans statins decrease triglycerides as well as total cholesterol, especially

LDL (Conde *et al.*, 1999; Streja *et al.*, 2002). We have no explanation for this diet-dependent response in triglycerides during pravastatin and *C. pneumoniae*. Generally, infection studies have been carried out in genetically modified, i.e. more "humanized" mice, such as heterozygous LDL receptor (LDLR) +/- and apolipoprotein E (apoE) +/- mouse strains. These mice are more sensitive to dietary lipids, and significant changes in lipoprotein profiles during infection could thus be expected. Our data highlight the importance of using a suitable animal model to study lipid/lipoprotein changes during infection.

## 5.3 Novel enzymeimmunoassay utilizing lipopolysaccharide binding protein as a capture molecule for the measurement of chlamydial lipopolysaccharide in serum (III)

### 5.3.1 Optimization of EIA conditions

D e t e r g e n t s

In serum, LPS can be found in several compartments, incorporated into lipoprotein membranes, complexed with immunoglobulins (especially IgM), associated with carrier or receptor proteins (LBP, mCD14, sCD14) or as a free micellar form. To optimize LPS dissociation conditions, we tested a set of detergents with different physicochemical natures. Among them, sodium cholate, Brij 35 and octylglucoside (in PBS) were found to be the most effective (Fig. 7).

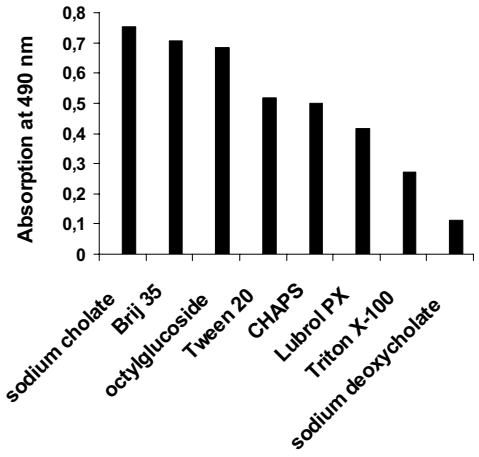


**Fig. 7.** The ability of detergents to solubilize *C. pneumoniae* LPS. Serum samples spiked with *C. pneumoniae* K6 elementary bodies (EBs) were examined by using 96-well microtiter plates coated with a catching monoclonal anti-lipopolysaccharide-binding protein (mouse anti-LBP) antibody (0.5 µg/well). Plates were first incubated with human LBP (1 µg/ml) for one hour at +37 ºC. After washing, serum containing *C. pneumoniae* K6 EBs in the presence of different detergents was added and incubated for one hour at +37 ºC. The plates were washed, and a biotinylated mouse monoclonal anti-cLPS antibody (Spriggs *et al.*) was used for detection.

Sodium cholate, Brij 35 and octylglucoside were further studied at 0.1 % and 0.5 % concentrations. A better resolution with each detergent was obtained at a 0.1 % concentration. Based on the sensitivity at low cLPS concentrations, a 0.1 % non-ionic octylglucoside was chosen for the sample pre-treatment.

Due to lipophilic and hydrophilic moieties, LPS exists in several aggregated forms in aqueous preparations and, most crucially, is in the biologically active state as a monomer (Mukerjee *et al.*, 1999). For this purpose, detergents are indispensable in solubilising LPS from a variety of serum complexes. In our assay, we used LBP as a natural binding agent for the lipid A part of the LPS molecule and monoclonal antibodies against a KDO-oligosaccharide epitope of LPS. Under these conditions, the detergent used in an EIA assay must preserve the functional and structural properties of LPS. We are aware that octylgucoside at a 0.1 % concentration is far below the critical micelle concentration (CMC) determined for this detergent (Neugebauer, 1990). Concerning membrane proteins, operating below CMC has been suggested to limit the concentration range at which solubilization progresses (le Maire *et al.*, 2000). However, experiments with 0.5 % octylglucoside, very close to its CMC, yielded a poor response in the LPS-EIA assay. On the other hand, octylglucoside at concentrations above CMC were not studied.

### Coating antibody and blocking buffer

Mouse moclonal anti-LPS antibody clone 6703 reacted with *Salmonella minnesota* re-LPS. Mab clones 6701 and 6709 were included for further analysis. Mab clone 6709 showed a more efficient cLPS binding capacity than clone 6701. The optimum LPS response was achieved at a 0.5 µg/ml coating antibody concentration. To avoid non-specific binding, the EIA response for cLPS with the 6709 antibody was then investigated in the presence of various blocking buffers. The most optimal antigen-antibody reaction and minimal background was reached by blocking plates with PBS supplemented with 0.2 % defatted milk.

In our EIA assay, *Chlamydia*-specificity was determined by a using monoclonal anti-cLPS antibody, clone 6709, as a trapping agent. This antibody showed no reactivity with *Bartonella henselae* or *Simkania negevensis*, which have previously been demonstrated to either cross-react (Maurin *et al.*, 1997) or display structural similarity with *Chlamydia* (Lieberman *et al.*, 2002). Moreover, the capture antibody did not bind *S. minnesota* Re-LPS, structurally and immunologically related to cLPS (Brade *et al.*, 1985; Brade *et al.*, 1986; Nurminen *et al.*, 1984). Hence, coating antibody 6709 was *Chlamydia*-specific and recognizes either the disaccharide (α–KDO

(2→8)-α-KDO) or trisaccharide (α-KDO-(2→8)-α-KDO (2→4)-α-KDO) part of KDO in the cLPS structure (Brade *et al.*, 1994). Finally, since cLPS is a group-specific antigen in *Chlamydia* (Dhir *et al.*, 1972), our EIA method can be used for measurement of chlamydial LPS without discrimination between chlamydial species. Nevertheless, it is evident that in genital infections, the amount of LPS in the lesions is too low to be detectable in serum. However, in ascending complications of *C. trachomatis* infections, such as pelvic inflammatory disease and perihepatitis, cLPS could be present in serum (Puolakkainen *et al.*, 1990) and give a positive result in the present cLPS-EIA test. The current EIA would be most suitable for diagnosis of chronic *C. pneumoniae* infection in the lungs or in the vasculature. These diseases are common in the elderly, in which *C. trachomatis* infections are rare. Use of cLPS-EIA in the diagnosis of acute infections might also be possible.

### Other EIA components

Optimal dilutions of LBP, a detection antibody (a-LBP) as well as an enzyme-labeled secondary antibody (GAR-HRP) were defined by checkerboard titration. The maximum EIA signal for cLPS was achieved with the following combination: LBP 1 µg/ml, polyclonal a-LBP 1:500, both diluted in 0.1 % Tween 20 and GAR-HRP 1:10 000 in Tween 20-PBS supplemented with 10 % fetal bovine serum (FBS).

Solubilization and monomerization of cLPS from all serum complexes is a prerequisite for the reliable quantitation of LPS by the EIA method. The novelty to the present assay comes from the utilization of a natural LPS-binding protein, LBP, as an LPS capture agent before trapping LPS by a solid phase monoclonal antibody. Given that the LBP concentration in normal human serum is about 5-10 µg/ml, we used a huge excess of LBP (1 µg/ml of LBP for sera diluted 1:50) in the assay. Since a lower LBP concentration (0.5 µg/ml) resulted in a weaker response, significant excess may be necessary to dissolve LPS from complex forms in serum.

### Standardization of the assay

In order to standardize the EIA assay, cLPS was purified with the phenol/chloroform extraction method (Rund *et al.*, 1999) from a *C. pneumoniae* K6 isolate. Purified cLPS was analyzed by 15 % SDS-PAGE and visualized by silver staining (Lee *et al.*, 1989). cLPS displayed a single, 10-15 kDa band (III, Fig. 2). Approximately the same size for cLPS has been reported earlier (Nurminen *et al.*, 1983; Nurminen *et al.*, 1984). A KDO-thiobarbituric assay was used for measuring the cLPS concentration (Waravdekar *et al.*, 1959), which was 84,1 µg/ml in the extract. When EIA was standardized with purified cLPS, a concentration-dependent response was obtained (III, Fig. 3).

### Final procedure and application of the method for clinical material

After optimizing all EIA conditions, the final procedure described in Chapter 4.2.3 (page 41) was attained and used for clinical applications. The EIA consisted of four incubation steps and finally, a relatively large immunological complex was built (III, Fig. 1). Nevertheless, multiple incubation steps are known to improve EIA sensitivity and therefore, a reduction in the number of incubation steps was not required. This newly developed EIA was first applied to serum cLPS quantification from 102 ACS patients and their age- and sex-matched controls (III, Table 1). The criteria for ACS, originally described elsewhere (Sinisalo et al., 2002), are briefly described in Chapter 4.1.2. Significantly higher cLPS prevalence and concentrations were found in ACS patients compared to healthy controls (III, Table 1; $P < 0.001$ for both). Analysis within genders revealed that the difference in cLPS positivity among women was not significantly different although the cLPS concentration was still significantly higher in ACS patients than in the controls (III, Table 1). This result could be related to the small number of women studied. Analysis of the whole data with an ROC curve, commonly used in evaluating the diagnostic value of the method, indicated nearly 80 % sensitivity and specificity for the cLPS-EIA to separate ACS patients from their control subjects [III, Fig. 4, area under the curve (AUC) value 0.797].

The diagnosis of chronic *C. pneumoniae* infection is problematic. After an acute infection, IgM and IgA antibodies usually disappear in some months, while *C. pneumoniae* IgG antibodies may remain elevated for years (Domeika *et al.*, 1997; Paldanius *et al.*, 2005) and their presence can therefore be considered only a marker of present or past exposure to *C. pneumoniae*. Because of their shorter biological half-life, IgA antibodies are considered a more reliable marker of chronic infections (Saikku, 1992). PCR can be used for the detection of *C. pneumoniae* DNA in circulating white blood cells, but this approach requires special techniques not available in all laboratories. In addition, the detection of *C. pneumoniae* by PCR seems to lack sensitivity and needs further optimization (Smieja *et al.*, 2002). Overall, the need for direct diagnostic method for the chronic *C. pneumoniae* infection has grown.

In the present study, we developed a specific and sensitive EIA method for the quantification of cLPS utilizing a novel methodological approach. Since chronic *C. pneumoniae* infection has been strongly associated with cardiovascular diseases, the EIA was first applied to analyze cLPS levels among acute coronary syndrome patients and their controls. Serum cLPS was found more often and cLPS levels were also significantly higher in the ACS patients compared to healthy controls. Since the patient sera were collected after the cardiac event, large amounts of cLPS might have been liberated into circulation at the time of tissue damage. Supporting this,

earlier studies on cLPS immune complexes have suggested the formation of cLPS containing immune-complexes after a myocardial infarction in the presence of cLPS excess (Leinonen *et al.*, 1990). The evaluation of diagnostic efficacy and the clinical usefulness of the cLPS-EIA method with an ROC curve revealed high specificity and sensitivity to discriminate between ACS patients and controls. Further studies are needed to illuminate whether circulating cLPS is usable as a marker of increased risk for cardiac events.

## 5.4 Chlamydial lipopolysaccharide is present in serum during acute coronary syndrome and correlates with CRP levels (IV)

Markers of *C. pneumoniae* infection (*C. pneumoniae* IgA and cLPS levels) and hsCRP concentration were analysed in sera collected from ACS patients during a one-year follow-up period (for sample collection, see Chapter 4.1.2). The clinical history of the patients revealed hypertension, diabetes, and hypercholesterolemia in 42 %, 19 %, and 77 % of the subjects, respectively. In addition, 41 % of the patients were under statin therapy. Serum total cholesterol and LDL cholesterol concentrations were not significantly elevated (IV, Table 1). Acute myocardial infarctions (AMI) caused hospitalization in 71 % of the patients, the rest (29 %) entered hospital due to unstable angina (UA). The use of statins did not significantly affect serum cLPS or hsCRP levels (IV, Table 2). Except for hsCRP, some sporadic correlations of different clinical and laboratory parameters with cLPS were detected (IV, Table 2). Serum hsCRP and cLPS levels correlated significantly in the patients throughout the one-year follow-up period (IV, Fig 1), and were significantly elevated in AMI patients at the time of hospitalization when compared to those in UA patients. The highest hsCRP and cLPS levels were observed within 48 hours after the acute event (IV, Fig 1). Both hsCRP and cLPS levels started to decrease immediately after the ACS event till the end of the follow-up (IV, Fig. 1). However, *C. pneumoniae* IgA antibodies, detected in 53 % of the ACS patients, did not decline in parallel with cLPS during the follow-up (Fig. 8). Compared to control subjects, *C. pneumoniae* antibodies and cLPS levels were significantly elevated. It is well known that *C. pneumoniae* antibody prevalence increases with age (Grayston *et al.*, 1990; Hammerschlag, 2002). However, although the controls were not fully age-matched (mean ages of 64 and 57 years in the patients and controls, respectively), the small difference in age had no significant effects on the results.

LPS is one of the major components in the *C. pneumoniae* outer membrane. Chlamydial LPS is capable of inducing the inflammatory cytokine response even though its endotoxin activity has been shown to be significantly lower than that of enterobacterial LPS (Ingalls *et al.*, 1995). Despite its low potency to induce cytokine secretion, LPS seems to be the main chlamydial component to induce macrophage transformation to cholesterol-containing foam cells (Kalayoglu *et al*., 1998a). Chlamydial LPS is abundantly detectable in inoculated arteries over one month post *C. pneumoniae* infection in an *ex vivo* culture model (Poppert *et al.*, 2005). Furthermore, E. coli LPS has been demonstrated to stimulate CRP production *in vitro* (Calabro *et al.*, 2003). In the present study, a very significant correlation between

cLPS and CRP levels was demonstrated in the sera obtained from patients with ACS. After the coronary event, both cLPS and CRP levels decreased, and the correlation remained significant during the entire follow-up period. These results strongly suggest the liberation of cLPS from the damaged *C. pneumoniae*-infected arterial tissue site during an acute coronary event, which leads to dose-dependent production of CRP. Interestingly, *C. pneumoniae* IgA antibody titers stayed at a stable level during the follow-up (Fig. 8) and hence, did not reflect the actual behavior of the infection.

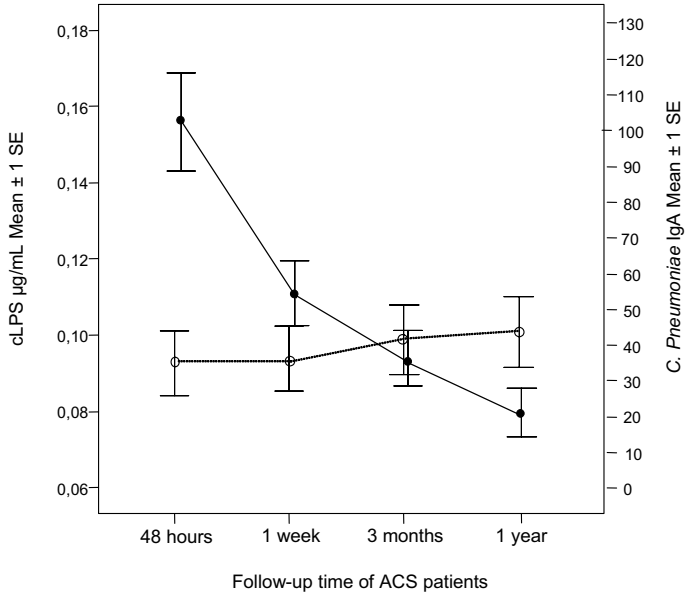


**Fig. 8.** Serum *C. pneumoniae* IgA antibody (open circle) and cLPS (closed circle) levels in ACS patients during the follow-up time.

Some earlier studies also postulate that high chlamydial LPS levels are present in circulation after an acute coronary event: acute myocardial infarction (AMI) patients show an antibody response to chlamydial LPS (Kiss *et al*., 2001; Saikku *et al.*,

1988), albeit IgA antibodies against *C. pneumoniae* structural proteins remain constantly elevated (Saikku *et al*., 1988). Consistent with these serological findings, the highest cLPS levels in ACS patients were observed during the acute event, suggesting the antigen is released from the ruptured lesion area. Reflecting the severity of the acute event, serum cLPS was significantly higher in patients with AMI as compared to those with UA. In both patient groups, cLPS levels decreased remarkably within one week of the event. Supporting this, previous observations demonstrate that in the acute phase of AMI, patients have a serum cLPS excess, which disappears within one month (Leinonen *et al.*, 1990). These data suggest relatively rapid clearance of cLPS from circulation after the AMI and agrees with the present finding, which shows decreased cLPS levels as early as one week after the ACS. Whether the combination of increased cLPS and hsCRP could be used as a risk marker for cardiac events and in monitoring the risk during intervention trials remains an interesting open question and needs further evaluation. Recent antibiotic intervention trials have shown no effect on *C. pneumoniae* infection (Anderson, 2005; Grayston *et al.*, 2005), suggesting that chronic *C. pneumoniae* infection can not be eradicated by monotherapy only, documented also in animal models (Rothstein *et al.*, 2001; Törmakangas *et al.*, 2004). Therefore, new generation antibiotics or relevant drug combinations may be needed to eliminate *C. pneumoniae* from the lesions and retard the progression of atherosclerosis.

The present data suggest that *C. pneumoniae,* being present in the lesions, may be involved in plaque rupture. The combination of hsCRP and cLPS quantitation in subjects might be a useful tool in the evaluation of the risk for cardiovascular events.

# 6 SUMMARY AND CONCLUSIONS

The first part of the study investigated the modulatory effects of acute *C. pneumoniae* infection alone and combined with statin treatment on infection, inflammation and lipid metabolism using an NIH/S mouse model. The focus in the latter part of the thesis was on chronic *C. pneumoniae* infection in human subjects. A new diagnostic method for *C. pneumoniae* was developed and the applicability of the method for analyzing clinical material was examined.

Although endotoxin administration in mice and in other rodents is widely used to mimic infection and results in more robust changes in lipid metabolism, intranasal infection with *C. pneumoniae* is probably a more physiologically relevant way to study the consequences of infection. Very mild changes in serum lipid metabolism were detected after a single *C. pneumoniae* inoculation in NIH/S mice with a transient increase in triglycerides and a decrease in LCAT activity. However, if these changes are prolonged during a chronic state, they may impair lipid metabolism and alter lipid distribution towards a pro-atherogenic profile. Regarding the effects of *C. pneumoniae* infection on serum lipid levels, other animal models with a lipid status similar to that detected in humans would be more functional.

The pulmonary inflammatory response to *C. pneumoniae* infection was accelerated by pravastatin treament compared to untreated control mice. However, no effect on *C. pneumoniae* growth could be demonstrated. Pravastatin induced unexpected changes in serum lipid levels during high fat. In uninfected and *C. pneumoniae* infected NIH/S mice, a significant increase in serum triglycerides was observed. A favourable, antiatherogenic lipid profile with the majority of the cholesterol in the HDL fraction may be one reason for the minor effect of pravastatin on cholesterol in NIH/S mice. Because mice are HDL mammals, it would be practical to study other animal models to discover whether pravastatin or other statins possess lipid-lowering or anti-inflammatory effects during *C. pneumoniae* infection.

A novel diagnostic method for *C. pneumoniae* infection was developed and applied to the serum material collected from ACS patients and their controls. The choice of detergent and optimization of all assay conditions yielded a specific and sensitive EIA method for cLPS quantification. The crucial point and novel approach was the use of LBP in LPS trapping from serum complexes. We found a significantly higher cLPS concentration and prevalence in ACS patients than in their controls. Evaluation of the method indicated as high as 80 % specificity and sensitivity in discriminating patients and controls in this setup. The correlation between hsCRP and cLPS suggested cLPS to be responsible for the inflammatory response. After

the acute coronary event, cLPS levels started to decrease within one week indicating i) the contribution of cLPS to an acute coronary event and ii) relatively rapid cLPS metabolism after ACS. Indicating poor diagnostic value, serum IgA antibodies were elevated in 53 % of the patients and remaining constant throughout the follow-up. The suitability of cLPS quantitation by the novel EIA method in other clinical and physiological settings remains a future challenge. Whether the combination of hsCRP and cLPS quantitation is a useful tool in the evaluation of the risk for cardio-vascular events should be verified in future studies.

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Tuusula, October 2006 Terttu Tiirola

# 8 REFERENCES


AALTO-SETÄLÄ, K., LAITINEN, K., ERKKILÄ, L., LEINONEN, M., JAUHIAINEN, M., EHNHOLM, C., TAMMINEN, M., PUOLAKKAINEN, M., PENTTILÄ, I. & SAIKKU, P. (2001). Chlamydia pneumoniae does not increase atherosclerosis in the aortic root of apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*, **21,** 578-84.

AIRENNE, S., SURCEL, H.M., ALAKÄRPPÄ, H., LAITINEN, K., PAAVONEN, J., SAIKKU, P. & LAURILA, A. (1999). Chlamydia pneumoniae infection in human monocytes. *Infect Immun*, **67,** 1445-9.

ALBERT, M.A., DANIELSON, E., RIFAI, N. & RIDKER, P.M. (2001). Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. *Jama*, **286,** 64-70.

ALVAREZ, C. & RAMOS, A. (1986). Lipids, lipoproteins, and apoproteins in serum during infection. *Clin Chem*, **32,** 142-5.

ANDERSON, J.L. (2005). Infection, antibiotics, and atherothrombosis--end of the road or new beginnings? *N Engl J Med*, **352,** 1706-9.

APFALTER, P., BLASI, F., BOMAN, J., GAYDOS, C.A., KUNDI, M., MAASS, M., MAKRISTATHIS, A., MEIJER, A., NADRCHAL, R., PERSSON, K., ROTTER, M.L., TONG, C.Y., STANEK, G. & HIRSCHL, A.M. (2001). Multicenter comparison trial of DNA extraction methods and PCR assays for detection of Chlamydia pneumoniae in endarterectomy specimens. *J Clin Microbiol*, **39,** 519-24.

AVIRAM, M., HARDAK, E., VAYA, J., MAHMOOD, S., MILO, S., HOFFMAN, A., BILLICKE, S., DRAGANOV, D. & ROSENBLAT, M. (2000). Human serum paraoxonases (PON1) Q and R selectively decrease lipid peroxides in human coronary and carotid atherosclerotic lesions: PON1 esterase and peroxidase-like activities. *Circulation*, **101,** 2510-7.

BAKKER-ARKEMA, R.G., DAVIDSON, M.H., GOLDSTEIN, R.J., DAVIGNON, J., ISAACSOHN, J.L., WEISS, S.R., KEILSON, L.M., BROWN, W.V., MILLER, V.T., SHURZINSKE, L.J. & BLACK, D.M. (1996). Efficacy and safety of a new HMG-CoA reductase inhibitor, atorvastatin, in patients with hypertriglyceridemia. *Jama*, **275,** 128-33.

BANNANTINE, J.P., GRIFFITHS, R.S., VIRATYOSIN, W., BROWN, W.J. & ROCKEY, D.D. (2000). A secondary structure motif predictive of protein localization to the chlamydial inclusion membrane. *Cell Microbiol*, **2,** 35-47.

BANNANTINE, J.P., ROCKEY, D.D. & HACKSTADT, T. (1998). Tandem genes of Chlamydia psittaci that encode proteins localized to the inclusion membrane. *Mol Microbiol*, **28,** 1017-26.

BARANOVA, I., VISHNYAKOVA, T., BOCHAROV, A., CHEN, Z., REMALEY, A.T., STONIK, J., EGGERMAN, T.L. & PATTERSON, A.P. (2002). Lipopolysaccharide down

regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun*, **70,** 2995-3003.

BARBOUR, A.G., AMANO, K., HACKSTADT, T., PERRY, L. & CALDWELL, H.D. (1982). Chlamydia trachomatis has penicillin-binding proteins but not detectable muramic acid. *J Bacteriol*, **151,** 420-8.

BARLAGE, S., FROHLICH, D., BOTTCHER, A., JAUHIAINEN, M., MULLER, H.P., NOETZEL, F., ROTHE, G., SCHUTT, C., LINKE, R.P., LACKNER, K.J., EHNHOLM, C. & SCHMITZ, G. (2001). ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response. *J Lipid Res*, **42,** 281-90.

BARRANS, A., COLLET, X., BARBARAS, R., JASPARD, B., MANENT, J., VIEU, C., CHAP, H. & PERRET, B. (1994). Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2. A study comparing liver perfusion to in vitro incubation with lipases. *J Biol Chem*, **269,** 11572-7.

BARTER, P., KASTELEIN, J., NUNN, A. & HOBBS, R. (2003). High density lipoproteins (HDLs) and atherosclerosis; the unanswered questions. *Atherosclerosis*, **168,** 195-211.

BARTER, P.J. & RYE, K.A. (1999). Lecithin:cholesterol acyltransferase. In *Lipoprotein in health and disease*. eds Betteridge, D.J., Illingworth, D.R. & Shepherd, J. London: Arnold.

BEATTY, W.L., BELANGER, T.A., DESAI, A.A., MORRISON, R.P. & BYRNE, G.I. (1994a). Role of tryptophan in gamma interferon-mediated chlamydial persistence. *Ann N Y Acad Sci*, **730,** 304-6.

BEATTY, W.L., BELANGER, T.A., DESAI, A.A., MORRISON, R.P. & BYRNE, G.I. (1994b). Tryptophan depletion as a mechanism of gamma interferon-mediated chlamydial persistence. *Infect Immun*, **62,** 3705-11.

BELLAND, R.J., OUELLETTE, S.P., GIEFFERS, J. & BYRNE, G.I. (2004). Chlamydia pneumoniae and atherosclerosis. *Cell Microbiol*, **6,** 117-27.

BLAIRON, L., WITTEBOLE, X. & LATERRE, P.F. (2003). Lipopolysaccharide-binding protein serum levels in patients with severe sepsis due to gram-positive and fungal infections. *J Infect Dis*, **187,** 287-91.

BLESSING, E., CAMPBELL, L.A., ROSENFELD, M.E., CHOUGH, N. & KUO, C.C. (2001). Chlamydia pneumoniae infection accelerates hyperlipidemia induced atherosclerotic lesion development in C57BL/6J mice. *Atherosclerosis*, **158,** 13-7.

BLESSING, E., CAMPBELL, L.A., ROSENFELD, M.E. & KUO, C.C. (2002). Chlamydia pneumoniae and hyperlipidemia are co-risk factors for atherosclerosis: infection prior to induction of hyperlipidemia does not accelerate development of atherosclerotic lesions in C57BL/6J mice. *Infect Immun*, **70,** 5332-4.

BLESSING, E., LIN, T.M., CAMPBELL, L.A., ROSENFELD, M.E., LLOYD, D. & KUO, C. (2000). Chlamydia pneumoniae induces inflammatory changes in the heart and aorta of normocholesterolemic C57BL/6J mice. *Infect Immun*, **68,** 4765-8.

BOMAN, J. & HAMMERSCHLAG, M.R. (2002). Chlamydia pneumoniae and atherosclerosis: critical assessment of diagnostic methods and relevance to treatment studies. *Clin Microbiol Rev*, **15,** 1-20.

BOMAN, J., SODERBERG, S., FORSBERG, J., BIRGANDER, L.S., ALLARD, A., PERSSON, K., JIDELL, E., KUMLIN, U., JUTO, P., WALDENSTROM, A. & WADELL, G. (1998). High prevalence of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells in patients with cardiovascular disease and in middle-aged blood donors. *J Infect Dis*, **178,** 274-7.

BRADE, H., BRADE, L. & NANO, F.E. (1987). Chemical and serological investigations on the genus-specific lipopolysaccharide epitope of Chlamydia. *Proc Natl Acad Sci U S A*, **84,** 2508-12.

BRADE, L., BRUNNEMANN, H., ERNST, M., FU, Y., HOLST, O., KOSMA, P., NAHER, H., PERSSON, K. & BRADE, H. (1994). Occurrence of antibodies against chlamydial lipopolysaccharide in human sera as measured by ELISA using an artificial glycoconjugate antigen. *FEMS Immunol Med Microbiol*, **8,** 27-41.

BRADE, L., NURMINEN, M., MäKELÄ, P.H. & BRADE, H. (1985). Antigenic properties of Chlamydia trachomatis lipopolysaccharide. *Infect Immun*, **48,** 569-72.

BRADE, L., SCHRAMEK, S., SCHADE, U. & BRADE, H. (1986). Chemical, biological, and immunochemical properties of the Chlamydia psittaci lipopolysaccharide. *Infect Immun*, **54,** 568-74.

BRAUN, J., LAITKO, S., TREHARNE, J., EGGENS, U., WU, P., DISTLER, A. & SIEPER, J. (1994). Chlamydia pneumoniae--a new causative agent of reactive arthritis and undifferentiated oligoarthritis. *Ann Rheum Dis*, **53,** 100-5.

BRAUNWALD, E. (1997). Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med*, **337,** 1360-9.

BROWN, M.S. & GOLDSTEIN, J.L. (1986). A receptor-mediated pathway for cholesterol homeostasis. *Science*, **232,** 34-47.

BROWN, M.S. & GOLDSTEIN, J.L. (1997). The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell*, **89,** 331-40.

CABANA, V.G., REARDON, C.A., FENG, N., NEATH, S., LUKENS, J. & GETZ, G.S. (2003). Serum paraoxonase: effect of the apolipoprotein composition of HDL and the acute phase response. *J Lipid Res*, **44,** 780-92.

CABANA, V.G., REARDON, C.A., WEI, B., LUKENS, J.R. & GETZ, G.S. (1999). SAA-only HDL formed during the acute phase response in apoA-I+/+ and apoA-I-/- mice. *J Lipid Res*, **40,** 1090-103.

CABANA, V.G., SIEGEL, J.N. & SABESIN, S.M. (1989). Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J Lipid Res*, **30,** 39-49.

CALABRO, P., WILLERSON, J.T. & YEH, E.T. (2003). Inflammatory cytokines stimulated C-reactive protein production by human coronary artery smooth muscle cells. *Circulation*, **108,** 1930-2.

CALDWELL, H.D., KROMHOUT, J. & SCHACHTER, J. (1981). Purification and partial characterization of the major outer membrane protein of Chlamydia trachomatis. *Infect Immun*, **31,** 1161-76.

CALIGIURI, G., ROTTENBERG, M., NICOLETTI, A., WIGZELL, H. & HANSSON, G.K. (2001). Chlamydia pneumoniae infection does not induce or modify atherosclerosis in mice. *Circulation*, **103,** 2834-8.

CAMPBELL, L.A., KUO, C.C. & GRAYSTON, J.T. (1990). Structural and antigenic analysis of Chlamydia pneumoniae. *Infect Immun*, **58,** 93-7.

CAMPBELL, L.A., MOAZED, T.C., KUO, C.C. & GRAYSTON, J.T. (1998). Preclinical models for Chlamydia pneumoniae and cardiovascular disease: hypercholesterolemic mice. *Clin Microbiol Infect*, **4 Suppl 4,** S23-S32.

CAMPBELL, L.A., NOSAKA, T., ROSENFELD, M.E., YARAEI, K. & KUO, C.C. (2005). Tumor necrosis factor alpha plays a role in the acceleration of atherosclerosis by Chlamydia pneumoniae in mice. *Infect Immun*, **73,** 3164-5.

CAMPBELL, L.A., O'BRIEN, E.R., CAPPUCCIO, A.L., KUO, C.C., WANG, S.P., STEWART, D., PATTON, D.L., CUMMINGS, P.K. & GRAYSTON, J.T. (1995). Detection of Chlamydia pneumoniae TWAR in human coronary atherectomy tissues. *J Infect Dis*, **172,** 585-8.

CATRON, D.M., LANGE, Y., BORENSZTAJN, J., SYLVESTER, M.D., JONES, B.D. & HALDAR, K. (2004). Salmonella enterica serovar Typhimurium requires nonsterol precursors of the cholesterol biosynthetic pathway for intracellular proliferation. *Infect Immun*, **72,** 1036-42.

CHESEBRO, B.B., BLESSING, E., KUO, C.C., ROSENFELD, M.E., PUOLAKKAINEN, M. & CAMPBELL, L.A. (2003). Nitric oxide synthase plays a role in Chlamydia pneumoniae-induced atherosclerosis. *Cardiovasc Res*, **60,** 170-4.

CHOW, J.C., YOUNG, D.W., GOLENBOCK, D.T., CHRIST, W.J. & GUSOVSKY, F. (1999). Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem*, **274,** 10689-92.

CHRISTIANSEN, G., BOESEN, T., HJERNO, K., DAUGAARD, L., MYGIND, P., MADSEN, A.S., KNUDSEN, K., FALK, E. & BIRKELUND, S. (1999). Molecular biology of Chlamydia pneumoniae surface proteins and their role in immunopathogenicity. *Am Heart J*, **138,** S491-5.

CLAUS, R.A., RUSSWURM, S., DOHRN, B., BAUER, M. & LOSCHE, W. (2005). Plasma platelet-activating factor acetylhydrolase activity in critically ill patients. *Crit Care Med*, **33,** 1416-9.

CLES, L.D. & STAMM, W.E. (1990). Use of HL cells for improved isolation and passage of Chlamydia pneumoniae. *J Clin Microbiol*, **28,** 938-40.

CLIFTON, P.M., MACKINNON, A.M. & BARTER, P.J. (1985). Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoproteins in subjects with myocardial infarction. *J Lipid Res*, **26,** 1389-98.

COETZEE, G.A., STRACHAN, A.F., VAN DER WESTHUYZEN, D.R., HOPPE, H.C., JEENAH, M.S. & DE BEER, F.C. (1986). Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. *J Biol Chem*, **261,** 9644-51.

CONDE, K., PINEDA, G., NEWTON, R.S. & FERNANDEZ, M.L. (1999). Hypocholesterolemic effects of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors in the guinea pig: atorvastatin versus simvastatin. *Biochem Pharmacol*, **58,** 1209-19.

CONLAN, J.W., CLARKE, I.N. & WARD, M.E. (1988). Epitope mapping with solid-phase peptides: identification of type-, subspecies-, species- and genus-reactive antibody binding domains on the major outer membrane protein of Chlamydia trachomatis. *Mol Microbiol*, **2,** 673-9.

COX, R.A. & GOKCEN, M. (1975). Effect of simian virus 40 subcutaneous tumors on circulating lipids and lipoproteins in the Syrian hamster. *J Natl Cancer Inst*, **54,** 379-86.

DECHEND, R., GIEFFERS, J., DIETZ, R., JOERRES, A., RUPP, J., LUFT, F.C. & MAASS, M. (2003). Hydroxymethylglutaryl coenzyme A reductase inhibition reduces Chlamydia pneumoniae-induced cell interaction and activation. *Circulation*, **108,** 261-5.

DHIR, S.P., HAKOMORI, S., KENNY, G.E. & GRAYSTON, J.T. (1972). Immunochemical studies on chlamydial group antigen (presence of a 2-keto-3-deoxycarbohydrate as immunodominant group). *J Immunol*, **109,** 116-22.

DIOMEDE, L., ALBANI, D., SOTTOCORNO, M., DONATI, M.B., BIANCHI, M., FRUSCELLA, P. & SALMONA, M. (2001). In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. *Arterioscler Thromb Vasc Biol*, **21,** 1327-32.

DITTRICH, R., DRAGONAS, C., MUELLER, A., MALTARIS, T., RUPP, J., BECKMANN, M.W. & MAASS, M. (2004). Endothelial Chlamydia pneumoniae infection promotes oxidation of LDL. *Biochem Biophys Res Commun*, **319,** 501-5.

DOMEIKA, K., BRADE, L., MARDH, P.A., BRADE, H., WITKIN, S.S. & DOMEIKA, M. (1997). Characterization of serum antibody response to chlamydiae in patients with sexually acquired reactive arthritis. *FEMS Immunol Med Microbiol*, **19,** 191-202.

DOWELL, S.F., PEELING, R.W., BOMAN, J., CARLONE, G.M., FIELDS, B.S., GUARNER, J., HAMMERSCHLAG, M.R., JACKSON, L.A., KUO, C.C., MAASS, M., MESSMER, T.O., TALKINGTON, D.F., TONDELLA, M.L. & ZAKI, S.R. (2001). Standardizing

Chlamydia pneumoniae assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clin Infect Dis*, **33,** 492-503.

DRAGANOV, D.I., STETSON, P.L., WATSON, C.E., BILLECKE, S.S. & LA DU, B.N. (2000). Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. *J Biol Chem*, **275,** 33435-42.

EAKER, E.D., PINSKY, J. & CASTELLI, W.P. (1992). Myocardial infarction and coronary death among women: psychosocial predictors from a 20-year follow-up of women in the Framingham Study. *Am J Epidemiol*, **135,** 854-64.

EISENBERG, S. (1984). High density lipoprotein metabolism. *J Lipid Res*, **25,** 1017-58.

EKMAN, M.R., GRAYSTON, J.T., VISAKORPI, R., KLEEMOLA, M., KUO, C.C. & SAIKKU, P. (1993a). An epidemic of infections due to Chlamydia pneumoniae in military conscripts. *Clin Infect Dis*, **17,** 420-5.

EKMAN, M.R., LEINONEN, M., SYRJÄLÄ, H., LINNANMÄKI, E., KUJALA, P. & SAIKKU, P. (1993b). Evaluation of serological methods in the diagnosis of Chlamydia pneumoniae pneumonia during an epidemic in Finland. *Eur J Clin Microbiol Infect Dis*, **12,** 756-60.

ENDO, A., TSUJITA, Y., KURODA, M. & TANZAWA, K. (1977). Inhibition of cholesterol synthesis in vitro and in vivo by ML-236A and ML-236B, competitive inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Eur J Biochem*, **77,** 31-6.

ENDO, S., INADA, K., YAMASHITA, H., TAKAKUWA, T., NAKAE, H., KASAI, T., KIKUCHI, M., OGAWA, M., UCHIDA, K. & YOSHIDA, M. (1994). Platelet-activating factor (PAF) acetylhydrolase activity, type II phospholipase A2, and cytokine levels in patients with sepsis. *Res Commun Chem Pathol Pharmacol*, **83,** 289-95.

ERKKILÄ, L., JAUHIAINEN, M., LAITINEN, K., HAASIO, K., TIIROLA, T., SAIKKU, P. & LEINONEN, M. (2005). Effect of Simvastatin, an Established Lipid-Lowering Drug, on Pulmonary Chlamydia pneumoniae Infection in Mice. *Antimicrob Agents Chemother*, **49,** 3959-62.

ERKKILÄ, L., LAITINEN, K., HAASIO, K., TIIROLA, T., JAUHIAINEN, M., LEHR, H.A., AALTO-SETÄLÄ, K., SAIKKU, P. & LEINONEN, M. (2004). Heat shock protein 60 autoimmunity and early lipid lesions in cholesterol-fed C57BL/6JBom mice during Chlamydia pneumoniae infection. *Atherosclerosis*, **177,** 321-8.

ERKKILÄ, L., LAITINEN, K., LAURILA, A., SAIKKU, P. & LEINONEN, M. (2002). Experimental Chlamydia pneumoniae infection in NIH/S mice: effect of reinoculation with chlamydial or cell preparation on culture, PCR and histological findings of lung tissue. *Vaccine*, **20,** 2318-24.

ERKKILÄ, L., ROTTENBERG, M.E. & LAITINEN, K. (2000). Comparison of anesthetics for inoculation of mice with Chlamydia pneumoniae. *Comp Med*, **50,** 46-8.

ERNTELL, M., LJUNGGREN, K., GADD, T. & PERSSON, K. (1989). Erythema nodosum--a manifestation of Chlamydia pneumoniae (strain TWAR) infection. *Scand J Infect Dis*, **21,** 693-6.

ETTINGER, W.H., VARMA, V.K., SORCI-THOMAS, M., PARKS, J.S., SIGMON, R.C., SMITH, T.K. & VERDERY, R.B. (1994). Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. *Arterioscler Thromb*, **14,** 8-13.

EVERETT, K.D., BUSH, R.M. & ANDERSEN, A.A. (1999). Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for the identification of organisms. *Int J Syst Bacteriol*, **49 Pt 2,** 415-40.

EVERETT, K.D. & HATCH, T.P. (1995). Architecture of the cell envelope of Chlamydia psittaci 6BC. *J Bacteriol*, **177,** 877-82.

EZZAHIRI, R., NELISSEN-VRANCKEN, H.J., KURVERS, H.A., STASSEN, F.R., VLIEGEN, I., GRAULS, G.E., VAN PUL, M.M., KITSLAAR, P.J. & BRUGGEMAN, C.A. (2002). Chlamydophila pneumoniae (Chlamydia pneumoniae) accelerates the formation of complex atherosclerotic lesions in Apo E3-Leiden mice. *Cardiovasc Res*, **56,** 269-76.

FEINGOLD, K.R., HARDARDOTTIR, I., MEMON, R., KRUL, E.J., MOSER, A.H., TAYLOR, J.M. & GRUNFELD, C. (1993a). Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. *J Lipid Res*, **34,** 2147-58.

FEINGOLD, K.R., KRAUSS, R.M., PANG, M., DOERRLER, W., JENSEN, P. & GRUNFELD, C. (1993b). The hypertriglyceridemia of acquired immunodeficiency syndrome is associated with an increased prevalence of low density lipoprotein subclass pattern B. *J Clin Endocrinol Metab*, **76,** 1423-7.

FEINGOLD, K.R., MEMON, R.A., MOSER, A.H., SHIGENAGA, J.K. & GRUNFELD, C. (1999). Endotoxin and interleukin-1 decrease hepatic lipase mRNA levels. *Atherosclerosis*, **142,** 379-87.

FEINGOLD, K.R., POLLOCK, A.S., MOSER, A.H., SHIGENAGA, J.K. & GRUNFELD, C. (1995). Discordant regulation of proteins of cholesterol metabolism during the acute phase response. *J Lipid Res*, **36,** 1474-82.

FEINGOLD, K.R., STAPRANS, I., MEMON, R.A., MOSER, A.H., SHIGENAGA, J.K., DOERRLER, W., DINARELLO, C.A. & GRUNFELD, C. (1992). Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J Lipid Res*, **33,** 1765-76.

FIELDING, C.J. & FIELDING, P.E. (1995). Molecular physiology of reverse cholesterol transport. *J Lipid Res*, **36,** 211-28.

FIERER, J., SWANCUTT, M.A., HEUMANN, D. & GOLENBOCK, D. (2002). The role of lipopolysaccharide binding protein in resistance to Salmonella infections in mice. *J Immunol*, **168,** 6396-403.

FISER, R.H., DENNISTON, J.C. & BEISEL, W.R. (1974). Endotoxemia in the Rhesus monkey: alterations in host lipid and carbohydrate metabolism. *Pediatr Res*, **8,** 13-7.

FISER, R.H., DENNISTON, J.C. & BEISEL, W.R. (1972). Infection with Diplococcus pneumoniae and Salmonella typhimurium in monkeys: changes in plasma lipids and lipoproteins. *J Infect Dis*, **125,** 54-60.

FISER, R.H., DENNISTON, J.C., RINDSIC, R.B. & BEISEL, W.R. (1971). Effects of acute infection on cholesterogenesis in the rhesus monkey. *Proc Soc Exp Biol Med*, **138,** 605-9.

FISER, R.H., SHULTZ, T.D., RINDSIG, R.B. & BEISEL, W.R. (1973). Alterations in plasma and brain lipid metabolism during endotoxemia in the neonatal rat. *Biol Neonate*, **22,** 155-60.

FITZGERALD, K.A., ROWE, D.C. & GOLENBOCK, D.T. (2004). Endotoxin recognition and signal transduction by the TLR4/MD2-complex. *Microbes Infect*, **6,** 1361-7.

FONG, I.W. (2000). Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. *Cmaj*, **163,** 49-56.

FONG, I.W., CHIU, B., VIIRA, E., FONG, M.W., JANG, D. & MAHONY, J. (1997). Rabbit model for Chlamydia pneumoniae infection. *J Clin Microbiol*, **35,** 48-52.

FOX, A., ROGERS, J.C., GILBART, J., MORGAN, S., DAVIS, C.H., KNIGHT, S. & WYRICK, P.B. (1990). Muramic acid is not detectable in Chlamydia psittaci or Chlamydia trachomatis by gas chromatography-mass spectrometry. *Infect Immun*, **58,** 835-7.

FREIDANK, H.M., LUX, A., DERN, P., MEYER-KONIG, U. & ELS, T. (2002). Chlamydia pneumoniae DNA in peripheral venous blood samples from patients with carotid artery stenosis. *Eur J Clin Microbiol Infect Dis*, **21,** 60-2.

FREY, E.A., MILLER, D.S., JAHR, T.G., SUNDAN, A., BAZIL, V., ESPEVIK, T., FINLAY, B.B. & WRIGHT, S.D. (1992). Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med*, **176,** 1665-71.

FRIEDLAND, M.L. & HERBERT, P.N. (1982). Lipoprotein abnormalities associated with a viral syndrome. *Jama*, **248,** 82.

FRIIS, R.R. (1972). Interaction of L cells and Chlamydia psittaci: entry of the parasite and host responses to its development. *J Bacteriol*, **110,** 706-21.

FROON, A.H., DENTENER, M.A., GREVE, J.W., RAMSAY, G. & BUURMAN, W.A. (1995). Lipopolysaccharide toxicity-regulating proteins in bacteremia. *J Infect Dis*, **171,** 1250-7.

FUKUSHI, H. & HIRAI, K. (1992). Proposal of Chlamydia pecorum sp. nov. for Chlamydia strains derived from ruminants. *Int J Syst Bacteriol*, **42,** 306-8.

GAAL, D., KREMMER, T., BALINT, Z., HOLCZINGER, L., BERTOK, L. & NOWOTNY, A. (1984). Effects of bacterial endotoxins and their detoxified derivatives on serum and liver lipids in mice. *Toxicol Appl Pharmacol*, **75,** 437-43.

GALLIN, J.I., KAYE, D. & O'LEARY, W.M. (1969). Serum lipids in infection. *N Engl J Med*, **281,** 1081-6.

GALLIN, J.I., O'LEARY, W.M. & KAYE, D. (1970). Serum concentrations of lipids in rabbits infected with Escherichia coli and Staphylococcus aureus. *Proc Soc Exp Biol Med*, **133,** 309-13.

GARRETT, A.J., HARRISON, M.J. & MANIRE, G.P. (1974). A search for the bacterial mucopeptide component, muramic acid, in Chlamydia. *J Gen Microbiol*, **80,** 315-8.

GAYDOS, C.A., SUMMERSGILL, J.T., SAHNEY, N.N., RAMIREZ, J.A. & QUINN, T.C. (1996). Replication of Chlamydia pneumoniae in vitro in human macrophages, endothelial cells, and aortic artery smooth muscle cells. *Infect Immun*, **64,** 1614-20.

GIEFFERS, J., VAN ZANDBERGEN, G., RUPP, J., SAYK, F., KRUGER, S., EHLERS, S., SOLBACH, W. & MAASS, M. (2004). Phagocytes transmit Chlamydia pneumoniae from the lungs to the vasculature. *Eur Respir J*, **23,** 506-10.

GIOANNINI, T.L., TEGHANEMT, A., ZHANG, D., COUSSENS, N.P., DOCKSTADER, W., RAMASWAMY, S. & WEISS, J.P. (2004). Isolation of an endotoxin-MD-2 complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations. *Proc Natl Acad Sci U S A*, **101,** 4186-91.

GODZIK, K.L., O'BRIEN, E.R., WANG, S.K. & KUO, C.C. (1995). In vitro susceptibility of human vascular wall cells to infection with Chlamydia pneumoniae. *J Clin Microbiol*, **33,** 2411-4.

GOLDBERG, I.J. (1996). Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res*, **37,** 693-707.

GORDON, B.R., PARKER, T.S., LEVINE, D.M., SAAL, S.D., WANG, J.C., SLOAN, B.J., BARIE, P.S. & RUBIN, A.L. (1996). Low lipid concentrations in critical illness: implications for preventing and treating endotoxemia. *Crit Care Med*, **24,** 584-9.

GORDON, T., CASTELLI, W.P., HJORTLAND, M.C., KANNEL, W.B. & DAWBER, T.R. (1977). Predicting coronary heart disease in middle-aged and older persons. The Framington study. *Jama*, **238,** 497-9.

GRAYSTON, J.T. (1989). Chlamydia pneumoniae, strain TWAR. *Chest*, **95,** 664-9.

GRAYSTON, J.T., ALDOUS, M.B., EASTON, A., WANG, S.P., KUO, C.C., CAMPBELL, L.A. & ALTMAN, J. (1993). Evidence that Chlamydia pneumoniae causes pneumonia and bronchitis. *J Infect Dis*, **168,** 1231-5.

GRAYSTON, J.T., CAMPBELL, L.A., KUO, C.C., MORDHORST, C.H., SAIKKU, P., THOM, D.H. & WANG, S.P. (1990). A new respiratory tract pathogen: Chlamydia pneumoniae strain TWAR. *J Infect Dis*, **161,** 618-25.

GRAYSTON, J.T., KRONMAL, R.A., JACKSON, L.A., PARISI, A.F., MUHLESTEIN, J.B., COHEN, J.D., ROGERS, W.J., CROUSE, J.R., BORROWDALE, S.L., SCHRON, E. & KNIRSCH, C. (2005). Azithromycin for the secondary prevention of coronary events. *N Engl J Med*, **352,** 1637-45.

GRAYSTON, J.T., KUO, C.C., COULSON, A.S., CAMPBELL, L.A., LAWRENCE, R.D., LEE, M.J., STRANDNESS, E.D. & WANG, S.P. (1995). Chlamydia pneumoniae (TWAR) in atherosclerosis of the carotid artery. *Circulation*, **92,** 3397-400.

GRAYSTON, J.T., KUO, C.C., WANG, S.P. & ALTMAN, J. (1986). A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med*, **315,** 161-8.

GRUBE, B.J., COCHANE, C.G., YE, R.D., GREEN, C.E., MCPHAIL, M.E., ULEVITCH, R.J. & TOBIAS, P.S. (1994). Lipopolysaccharide binding protein expression in primary human hepatocytes and HepG2 hepatoma cells. *J Biol Chem*, **269,** 8477-82.

GRUNFELD, C., KOTLER, D.P., SHIGENAGA, J.K., DOERRLER, W., TIERNEY, A., WANG, J., PIERSON, R.N., JR. & FEINGOLD, K.R. (1991). Circulating interferon-alpha levels and hypertriglyceridemia in the acquired immunodeficiency syndrome. *Am J Med*, **90,** 154-62.

GRUNFELD, C., PANG, M., DOERRLER, W., SHIGENAGA, J.K., JENSEN, P. & FEINGOLD, K.R. (1992). Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *J Clin Endocrinol Metab*, **74,** 1045-52.

GRÖNHAGEN-RISKA, C., SAIKKU, P., RISKA, H., FRÖSETH, B. & GRAYSTON, J.T. (1988). Antibodies to TWAR - a novel type of Chlamydia - in sarcoidosis. In *Sarcoidosis and Other Granulomatous Disorders.* ed Grassi, C.e.a. pp. 297-301. New York: Excerpta Medica.

HACKSTADT, T., SCIDMORE, M.A. & ROCKEY, D.D. (1995). Lipid metabolism in Chlamydia trachomatis-infected cells: directed trafficking of Golgi-derived sphingolipids to the chlamydial inclusion. *Proc Natl Acad Sci U S A*, **92,** 4877-81.

HAHN, D.L. (1992). Chlamydia pneumoniae infection and asthma. *Lancet*, **339,** 1173-4.

HAILMAN, E., ALBERS, J.J., WOLFBAUER, G., TU, A.Y. & WRIGHT, S.D. (1996a). Neutralization and transfer of lipopolysaccharide by phospholipid transfer protein. *J Biol Chem*, **271,** 12172-8.

HAILMAN, E., LICHENSTEIN, H.S., WURFEL, M.M., MILLER, D.S., JOHNSON, D.A., KELLEY, M., BUSSE, L.A., ZUKOWSKI, M.M. & WRIGHT, S.D. (1994). Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med*, **179,** 269-77.

HAILMAN, E., VASSELON, T., KELLEY, M., BUSSE, L.A., HU, M.C., LICHENSTEIN, H.S., DETMERS, P.A. & WRIGHT, S.D. (1996b). Stimulation of macrophages and neutrophils by complexes of lipopolysaccharide and soluble CD14. *J Immunol*, **156,** 4384-90.

HAMMERSCHLAG, M.R. (2002). Chlamydia pneumoniae and the heart: impact of diagnostic methods. *Curr Clin Top Infect Dis*, **22,** 24-41.

HARANAGA, S., YAMAGUCHI, H., FRIEDMAN, H., IZUMI, S. & YAMAMOTO, Y. (2001). Chlamydia pneumoniae infects and multiplies in lymphocytes in vitro. *Infect Immun*, **69,** 7753-9.

HARDARDOTTIR, I., GRUNFELD, C. & FEINGOLD, K.R. (1995). Effects of endotoxin on lipid metabolism. *Biochem Soc Trans*, **23,** 1013-8.

HARDARDOTTIR, I., MOSER, A.H., FULLER, J., FIELDING, C., FEINGOLD, K. & GRUNFELD, C. (1996). Endotoxin and cytokines decrease serum levels and extra hepatic protein and mRNA levels of cholesteryl ester transfer protein in syrian hamsters. *J Clin Invest*, **97,** 2585-92.

HARDARDOTTIR, I., MOSER, A.H., MEMON, R., GRUNFELD, C. & FEINGOLD, K.R. (1994). Effects of TNF, IL-1, and the combination of both cytokines on cholesterol metabolism in Syrian hamsters. *Lymphokine Cytokine Res*, **13,** 161-6.

HARDARDOTTIR, I., SIPE, J., MOSER, A.H., FIELDING, C.J., FEINGOLD, K.R. & GRUNFELD, C. (1997). LPS and cytokines regulate extra hepatic mRNA levels of apolipoproteins during the acute phase response in Syrian hamsters. *Biochim Biophys Acta*, **1344,** 210-20.

HARRIS, H.W., GRUNFELD, C., FEINGOLD, K.R. & RAPP, J.H. (1990). Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J Clin Invest*, **86,** 696-702.

HARRIS, H.W., GRUNFELD, C., FEINGOLD, K.R., READ, T.E., KANE, J.P., JONES, A.L., EICHBAUM, E.B., BLAND, G.F. & RAPP, J.H. (1993). Chylomicrons alter the fate of endotoxin, decreasing tumor necrosis factor release and preventing death. *J Clin Invest*, **91,** 1028-34.

HATCH, T.P., AL-HOSSAINY, E. & SILVERMAN, J.A. (1982). Adenine nucleotide and lysine transport in Chlamydia psittaci. *J Bacteriol*, **150,** 662-70.

HAVEKES, L., DE WIT, E., LEUVEN, J.G., KLASEN, E., UTERMANN, G., WEBER, W. & BEISIEGEL, U. (1986). Apolipoprotein E3-Leiden. A new variant of human apolipoprotein E associated with familial type III hyperlipoproteinemia. *Hum Genet*, **73,** 157-63.

HEINE, H., MULLER-LOENNIES, S., BRADE, L., LINDNER, B. & BRADE, H. (2003). Endotoxic activity and chemical structure of lipopolysaccharides from Chlamydia trachomatis serotypes E and L2 and Chlamydophila psittaci 6BC. *Eur J Biochem*, **270,** 440-50.

HIGASHI, N. (1965). Electron Microscopic Studies on the Mode of Reproduction of Trachoma Virus and Psittacosis Virus in Cell Cultures. *Exp Mol Pathol*, **76,** 24-39.

HIRSCH, R.L., MCKAY, D.G., TRAVERS, R.I. & SKRALY, R.K. (1964). Hyperlipidemia, Fatty Liver, and Bromsulfophthalein Retention in Rabbits Injected Intravenously with Bacterial Endotoxins. *J Lipid Res*, **53,** 563-8.

HOWARD, K.M., MILLER, J.E., MIWA, M. & OLSON, M.S. (1997). Cell-specific regulation of expression of plasma-type platelet-activating factor acetylhydrolase in the liver. *J Biol Chem*, **272,** 27543-8.

HU, H., PIERCE, G.N. & ZHONG, G. (1999). The atherogenic effects of chlamydia are dependent on serum cholesterol and specific to Chlamydia pneumoniae. *J Clin Invest*, **103,** 747-53.

HUITTINEN, T., LEINONEN, M., TENKANEN, L., VIRKKUNEN, H., MÄNTTÄRI, M., PALOSUO, T., MANNINEN, V. & SAIKKU, P. (2003). Synergistic effect of persistent Chlamydia pneumoniae infection, autoimmunity, and inflammation on coronary risk. *Circulation*, **107,** 2566-70.

HUSSEIN, A., SKULTETY, L. & TOMAN, R. (2001). Structural analyses of the lipopolysaccharides from Chlamydophila psittaci strain 6BC and Chlamydophila pneumoniae strain Kajaani 6. *Carbohydr Res*, **336,** 213-23.

HUUSKONEN, J., WOHLFAHRT, G., JAUHIAINEN, M., EHNHOLM, C., TELEMAN, O. & OLKKONEN, V.M. (1999). Structure and phospholipid transfer activity of human PLTP: analysis by molecular modeling and site-directed mutagenesis. *J Lipid Res*, **40,** 1123-30.

INGALLS, R.R., RICE, P.A., QURESHI, N., TAKAYAMA, K., LIN, J.S. & GOLENBOCK, D.T. (1995). The inflammatory cytokine response to Chlamydia trachomatis infection is endotoxin mediated. *Infect Immun*, **63,** 3125-30.

ISHIBASHI, S., BROWN, M.S., GOLDSTEIN, J.L., GERARD, R.D., HAMMER, R.E. & HERZ, J. (1993). Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest*, **92,** 883-93.

ISHIBASHI, S., HERZ, J., MAEDA, N., GOLDSTEIN, J.L. & BROWN, M.S. (1994). The two-receptor model of lipoprotein clearance: tests of the hypothesis in "knockout" mice lacking the low density lipoprotein receptor, apolipoprotein E, or both proteins. *Proc Natl Acad Sci U S A*, **91,** 4431-5.

JACK, R.S., FAN, X., BERNHEIDEN, M., RUNE, G., EHLERS, M., WEBER, A., KIRSCH, G., MENTEL, R., FURLL, B., FREUDENBERG, M., SCHMITZ, G., STELTER, F. & SCHUTT, C. (1997). Lipopolysaccharide-binding protein is required to combat a murine gram-negative bacterial infection. *Nature*, **389,** 742-5.

JAHN, H.U., KRULL, M., WUPPERMANN, F.N., KLUCKEN, A.C., ROSSEAU, S., SEYBOLD, J., HEGEMANN, J.H., JANTOS, C.A. & SUTTORP, N. (2000). Infection and activation of airway epithelial cells by Chlamydia pneumoniae. *J Infect Dis*, **182,** 1678-87.

JANSSON, E. (1960). Ornithosis in Helsinki and some other localities in Finland. A serological and clinical study. *Ann Med Exp Biol Fenn*, **38(Suppl 4),** 1-110.

JAUHIAINEN, M., METSO, J., PAHLMAN, R., BLOMQVIST, S., VAN TOL, A. & EHNHOLM, C. (1993). Human plasma phospholipid transfer protein causes high density lipoprotein conversion. *J Biol Chem*, **268,** 4032-6.

JERSMANN, H.P., HII, C.S., HODGE, G.L. & FERRANTE, A. (2001). Synthesis and surface expression of CD14 by human endothelial cells. *Infect Immun*, **69,** 479-85.

JIALAL, I., STEIN, D., BALIS, D., GRUNDY, S.M., ADAMS-HUET, B. & DEVARAJ, S. (2001). Effect of hydroxymethyl glutaryl coenzyme a reductase inhibitor therapy on high sensitive C-reactive protein levels. *Circulation*, **103,** 1933-5.

JIANG, X.C. & BRUCE, C. (1995). Regulation of murine plasma phospholipid transfer protein activity and mRNA levels by lipopolysaccharide and high cholesterol diet. *J Biol Chem*, **270,** 17133-8.

JIANG, X.C., BRUCE, C., MAR, J., LIN, M., JI, Y., FRANCONE, O.L. & TALL, A.R. (1999). Targeted mutation of plasma phospholipid transfer protein gene markedly reduces high-density lipoprotein levels. *J Clin Invest*, **103,** 907-14.

JOHNSTON, S.C., MESSINA, L.M., BROWNER, W.S., LAWTON, M.T., MORRIS, C. & DEAN, D. (2001). C-reactive protein levels and viable Chlamydia pneumoniae in carotid artery atherosclerosis. *Stroke*, **32,** 2748-52.

JOUSILAHTI, P., VARTIAINEN, E., TUOMILEHTO, J. & PUSKA, P. (1999). Sex, age, cardiovascular risk factors, and coronary heart disease: a prospective follow-up study of 14 786 middle-aged men and women in Finland. *Circulation*, **99,** 1165-72.

KALAYOGLU, M.V. & BYRNE, G.I. (1998a). A Chlamydia pneumoniae component that induces macrophage foam cell formation is chlamydial lipopolysaccharide. *Infect Immun*, **66,** 5067-72.

KALAYOGLU, M.V. & BYRNE, G.I. (1998b). Induction of macrophage foam cell formation by Chlamydia pneumoniae. *J Infect Dis*, **177,** 725-9.

KALAYOGLU, M.V., HOERNEMAN, B., LAVERDA, D., MORRISON, S.G., MORRISON, R.P. & BYRNE, G.I. (1999). Cellular oxidation of low-density lipoprotein by Chlamydia pneumoniae. *J Infect Dis*, **180,** 780-90.

KALAYOGLU, M.V., INDRAWATI, MORRISON, R.P., MORRISON, S.G., YUAN, Y. & BYRNE, G.I. (2000). Chlamydial virulence determinants in atherogenesis: the role of chlamydial lipopolysaccharide and heat shock protein 60 in macrophage-lipoprotein interactions. *J Infect Dis*, **181 Suppl 3,** S483-9.

KALAYOGLU, M.V., LIBBY, P. & BYRNE, G.I. (2002). Chlamydia pneumoniae as an emerging risk factor in cardiovascular disease. *Jama*, **288,** 2724-31.

KALAYOGLU, M.V., PERKINS, B.N. & BYRNE, G.I. (2001). Chlamydia pneumoniae-infected monocytes exhibit increased adherence to human aortic endothelial cells. *Microbes Infect*, **3,** 963-9.

KALMAN, S., MITCHELL, W., MARATHE, R., LAMMEL, C., FAN, J., HYMAN, R.W., OLINGER, L., GRIMWOOD, J., DAVIS, R.W. & STEPHENS, R.S. (1999). Comparative genomes of Chlamydia pneumoniae and C. trachomatis. *Nat Genet*, **21,** 385-9.

KARLSSON, L., GNARPE, J., NAAS, J., OLSSON, G., LINDHOLM, J., STEEN, B. & GNARPE, H. (2000). Detection of viable Chlamydia pneumoniae in abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg*, **19,** 630-5.

KAUFMANN, S.H. (1990). Heat shock proteins and the immune response. *Immunol Today*, **11,** 129-36.

KAUKORANTA-TOLVANEN, S.E., LAURILA, A.L., SAIKKU, P., LEINONEN, M. & LAITINEN, K. (1995). Experimental Chlamydia pneumoniae infection in mice: effect of reinfection and passive immunization. *Microb Pathog*, **18,** 279-88.

KAUKORANTA-TOLVANEN, S.S., LAITINEN, K., SAIKKU, P. & LEINONEN, M. (1994). Chlamydia pneumoniae multiplies in human endothelial cells in vitro. *Microb Pathog*, **16,** 313-9.

KAUKORANTA-TOLVANEN, S.S., LAURILA, A.L., SAIKKU, P., LEINONEN, M., LIESIROVA, L. & LAITINEN, K. (1993). Experimental infection of Chlamydia pneumoniae in mice. *Microb Pathog*, **15,** 293-302.

KAUKORANTA-TOLVANEN, S.S., TEPPO, A.M., LAITINEN, K., SAIKKU, P., LINNAVUORI, K. & LEINONEN, M. (1996). Growth of Chlamydia pneumoniae in cultured human peripheral blood mononuclear cells and induction of a cytokine response. *Microb Pathog*, **21,** 215-21.

KAUL, R., UPHOFF, J., WIEDEMAN, J., YADLAPALLI, S. & WENMAN, W.M. (2000). Detection of Chlamydia pneumoniae DNA in CD3+ lymphocytes from healthy blood donors and patients with coronary artery disease. *Circulation*, **102,** 2341-6.

KAWAKAMI, M., MURASE, T., ITAKURA, H., YAMADA, N., OHSAWA, N. & TAKAKU, F. (1986). Lipid metabolism in endotoxic rats: decrease in hepatic triglyceride lipase activity. *Microbiol Immunol*, **30,** 849-54.

KHOVIDHUNKIT, W., KIM, M.S., MEMON, R.A., SHIGENAGA, J.K., MOSER, A.H., FEINGOLD, K.R. & GRUNFELD, C. (2004). Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res*, **45,** 1169-96.

KHOVIDHUNKIT, W., MEMON, R.A., FEINGOLD, K.R. & GRUNFELD, C. (2000). Infection and inflammation-induced proatherogenic changes of lipoproteins. *J Infect Dis*, **181 Suppl 3,** S462-72.

KHOVIDHUNKIT, W., MEMON, R.A., SHIGENAGA, J.K., PANG, M., SCHAMBELAN, M., MULLIGAN, K., FEINGOLD, K.R. & GRUNFELD, C. (1999). Plasma platelet-activating factor acetylhydrolase activity in human immunodeficiency virus infection and the acquired immunodeficiency syndrome. *Metabolism*, **48,** 1524-31.

KHOVIDHUNKIT, W., SHIGENAGA, J.K., MOSER, A.H., FEINGOLD, K.R. & GRUNFELD, C. (2001). Cholesterol efflux by acute-phase high density lipoprotein: role of lecithin: cholesterol acyltransferase. *J Lipid Res*, **42,** 967-75.

KINLAY, S., SCHWARTZ, G.G., OLSSON, A.G., RIFAI, N., LESLIE, S.J., SASIELA, W.J., SZAREK, M., LIBBY, P. & GANZ, P. (2003). High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. *Circulation*, **108,** 1560-6.

KISILEVSKY, R. & TAM, S.P. (2002). Acute phase serum amyloid A, cholesterol metabolism, and cardiovascular disease. *Pediatr Pathol Mol Med*, **21,** 291-305.

KISS, K., KHANAKAH, G., KUNDI, M., GLOGAR, H.D. & STANEK, G. (2001). Increase of chlamydial LPS antibodies in patients with acute coronary syndrome without detection of chlamydial DNA in atherectomy samples. *Wien Klin Wochenschr*, **113,** 731-6.

KITCHENS, R.L. & MUNFORD, R.S. (1998). CD14-dependent internalization of bacterial lipopolysaccharide (LPS) is strongly influenced by LPS aggregation but not by cellular responses to LPS. *J Immunol*, **160,** 1920-8.

KITCHENS, R.L., THOMPSON, P.A., MUNFORD, R.S. & O'KEEFE, G.E. (2003). Acute inflammation and infection maintain circulating phospholipid levels and enhance lipopolysaccharide binding to plasma lipoproteins. *J Lipid Res*, **44,** 2339-48.

KLEINERT, H., SCHWARZ, P.M. & FORSTERMANN, U. (2003). Regulation of the expression of inducible nitric oxide synthase. *Biol Chem*, **384,** 1343-64.

KNAPP, S., DE VOS, A.F., FLORQUIN, S., GOLENBOCK, D.T. & VAN DER POLL, T. (2003). Lipopolysaccharide binding protein is an essential component of the innate immune response to Escherichia coli peritonitis in mice. *Infect Immun*, **71,** 6747-53.

KNUDSEN, K., MADSEN, A.S., MYGIND, P., CHRISTIANSEN, G. & BIRKELUND, S. (1999). Identification of two novel genes encoding 97- to 99-kilodalton outer membrane proteins of Chlamydia pneumoniae. *Infect Immun*, **67,** 375-83.

KOL, A., BOURCIER, T., LICHTMAN, A.H. & LIBBY, P. (1999). Chlamydial and human heat shock protein 60s activate human vascular endothelium, smooth muscle cells, and macrophages. *J Clin Invest*, **103,** 571-7.

KOL, A., SUKHOVA, G.K., LICHTMAN, A.H. & LIBBY, P. (1998). Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-alpha and matrix metalloproteinase expression. *Circulation*, **98,** 300-7.

KOZYRAKI, R., FYFE, J., KRISTIANSEN, M., GERDES, C., JACOBSEN, C., CUI, S., CHRISTENSEN, E.I., AMINOFF, M., DE LA CHAPELLE, A., KRAHE, R., VERROUST, P.J. & MOESTRUP, S.K. (1999). The intrinsic factor-vitamin B12 receptor, cubilin, is a high-affinity apolipoprotein A-I receptor facilitating endocytosis of high-density lipoprotein. *Nat Med*, **5,** 656-61.

KRULL, M., KRAMP, J., PETROV, T., KLUCKEN, A.C., HOCKE, A.C., WALTER, C., SCHMECK, B., SEYBOLD, J., MAASS, M., LUDWIG, S., KUIPERS, J.G., SUTTORP, N. & HIPPENSTIEL, S. (2004). Differences in cell activation by Chlamydophila pneumoniae and Chlamydia trachomatis infection in human endothelial cells. *Infect Immun*, **72,** 6615-21.

KUO, C.C., GOWN, A.M., BENDITT, E.P. & GRAYSTON, J.T. (1993a). Detection of Chlamydia pneumoniae in aortic lesions of atherosclerosis by immunocytochemical stain. *Arterioscler Thromb*, **13,** 1501-4.

KUO, C.C., GRAYSTON, J.T., CAMPBELL, L.A., GOO, Y.A., WISSLER, R.W. & BENDITT, E.P. (1995a). Chlamydia pneumoniae (TWAR) in coronary arteries of young adults (15-34 years old). *Proc Natl Acad Sci U S A*, **92,** 6911-4.

KUO, C.C., JACKSON, L.A., CAMPBELL, L.A. & GRAYSTON, J.T. (1995b). Chlamydia pneumoniae (TWAR). *Clin Microbiol Rev*, **8,** 451-61.

KUO, C.C., SHOR, A., CAMPBELL, L.A., FUKUSHI, H., PATTON, D.L. & GRAYSTON, J.T. (1993b). Demonstration of Chlamydia pneumoniae in atherosclerotic lesions of coronary arteries. *J Infect Dis*, **167,** 841-9.

KUTLIN, A., ROBLIN, P.M. & HAMMERSCHLAG, M.R. (1998). Antibody response to Chlamydia pneumoniae infection in children with respiratory illness. *J Infect Dis*, **177,** 720-4.

KWONG, L.K., RIDINGER, D.N., BANDHAUER, M., WARD, J.H., SAMLOWSKI, W.E., IVERIUS, P.H., PRITCHARD, H. & WILSON, D.E. (1997). Acute dyslipoproteinemia induced by interleukin-2: lecithin:cholesteryl acyltransferase, lipoprotein lipase, and hepatic lipase deficiencies. *J Clin Endocrinol Metab*, **82,** 1572-81.

LAITINEN, K., LAURILA, A.L., LEINONEN, M. & SAIKKU, P. (1996). Reactivation of Chlamydia pneumoniae infection in mice by cortisone treatment. *Infect Immun*, **64,** 1488-90.

LAMPING, N., DETTMER, R., SCHRODER, N.W., PFEIL, D., HALLATSCHEK, W., BURGER, R. & SCHUMANN, R.R. (1998). LPS-binding protein protects mice from septic shock caused by LPS or gram-negative bacteria. *J Clin Invest*, **101,** 2065-71.

LANDMANN, R., ZIMMERLI, W., SANSANO, S., LINK, S., HAHN, A., GLAUSER, M.P. & CALANDRA, T. (1995). Increased circulating soluble CD14 is associated with high mortality in gram-negative septic shock. *J Infect Dis*, **171,** 639-44.

LAURILA, A., BLOIGU, A., NäYHÄ, S., HASSI, J., LEINONEN, M. & SAIKKU, P. (1997). Chronic Chlamydia pneumoniae infection is associated with a serum lipid profile known to be a risk factor for atherosclerosis. *Arterioscler Thromb Vasc Biol*, **17,** 2910-3.

LAURILA, A.L., BLOIGU, A., NäYHÄ, S., HASSI, J., LEINONEN, M. & SAIKKU, P. (1998). Chlamydia pneumoniae antibodies associated with altered serum lipid profile. *Int J Circumpolar Health*, **57 Suppl 1,** 329-32.

LAVERDA, D. & BYRNE, G.I. (1997). Use of monoclonal antibodies to facilitate identification, cloning, and purification of Chlamydia trachomatis hsp10. *J Clin Microbiol*, **35,** 1209-15.

LE MAIRE, M., CHAMPEIL, P. & MOLLER, J.V. (2000). Interaction of membrane proteins and lipids with solubilizing detergents. *Biochim Biophys Acta*, **1508,** 86-111.

LEE, K.K. & ELLIS, A.E. (1989). Rapid and sensitive silver-lipopolysaccharide staining using PhastSystem in fast horizontal polyacrylamide gel electrophoresis. *Electrophoresis*, **10,** 729-31.

LEINONEN, M., LINNANMÄKI, E., MATTILA, K., NIEMINEN, M.S., VALTONEN, V., LEIRISALO-REPO, M. & SAIKKU, P. (1990). Circulating immune complexes containing chlamydial lipopolysaccharide in acute myocardial infarction. *Microb Pathog*, **9,** 67-73.

LEINONEN, M. & SAIKKU, P. (2002). Evidence for infectious agents in cardiovascular disease and atherosclerosis. *Lancet Infect Dis*, **2,** 11-7.

LEQUIRE, V.S., HUTCHERSON, J.D., HAMILTON, R.L. & GRAY, M.E. (1959). The effects of bacterial endotoxin on lipide metabolism. I. The responses of the serum lipides of rabbits to single and repeated injections of Shear's polysaccharide. *J Exp Med*, **110,** 293-309.

LEVELS, J.H., ABRAHAM, P.R., VAN DEN ENDE, A. & VAN DEVENTER, S.J. (2001). Distribution and kinetics of lipoprotein-bound endotoxin. *Infect Immun*, **69,** 2821-8.

LEVELS, J.H., MARQUART, J.A., ABRAHAM, P.R., VAN DEN ENDE, A.E., MOLHUIZEN, H.O., VAN DEVENTER, S.J. & MEIJERS, J.C. (2005). Lipopolysaccharide is transferred from high-density to low-density lipoproteins by lipopolysaccharide-binding protein and phospholipid transfer protein. *Infect Immun*, **73,** 2321-6.

LEVY, E., GURBINDO, C., LACAILLE, F., PARADIS, K., THIBAULT, L. & SEIDMAN, E. (1993). Circulating tumor necrosis factor-alpha levels and lipid abnormalities in patients with cystic fibrosis. *Pediatr Res*, **34,** 162-6.

LIEBERMAN, D., DVOSKIN, B., LIEBERMAN, D.V., KAHANE, S. & FRIEDMAN, M.G. (2002). Serological evidence of acute infection with the Chlamydia-like microorganism Simkania negevensis (Z) in acute exacerbation of chronic obstructive pulmonary disease. *Eur J Clin Microbiol Infect Dis*, **21,** 307-9.

LINNANMÄKI, E., LEINONEN, M., MATTILA, K., NIEMINEN, M.S., VALTONEN, V. & SAIKKU, P. (1993). Chlamydia pneumoniae-specific circulating immune complexes in patients with chronic coronary heart disease. *Circulation*, **87,** 1130-4.

LINSEL-NITSCHKE, P. & TALL, A.R. (2005). HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat Rev Drug Discov*, **4,** 193-205.

LIU, L., HU, H., JI, H., MURDIN, A.D., PIERCE, G.N. & ZHONG, G. (2000). Chlamydia pneumoniae infection significantly exacerbates aortic atherosclerosis in an LDLR-/- mouse model within six months. *Mol Cell Biochem*, **215,** 123-8.

LONGBOTTOM, D., RUSSELL, M., DUNBAR, S.M., JONES, G.E. & HERRING, A.J. (1998). Molecular cloning and characterization of the genes coding for the highly immunogenic cluster of 90-kilodalton envelope proteins from the Chlamydia psittaci subtype that causes abortion in sheep. *Infect Immun*, **66,** 1317-24.

LUDERITZ, O., GALANOS, C., RISSE, H.J., RUSCHMANN, E., SCHLECHT, S., SCHMIDT, G., SCHULTE-HOLTHAUSEN, H., WHEAT, R., WESTPHAL, O. & SCHLOSSHARDT, J. (1966a). Structural relationship of Salmonella O and R antigens. *Ann N Y Acad Sci*, **133,** 349-74.

LUDERITZ, O., STAUB, A.M. & WESTPHAL, O. (1966b). Immunochemistry of O and R antigens of Salmonella and related Enterobacteriaceae. *Bacteriol Rev*, **30,** 192-255.

LUKACOVA, M., BAUMANN, M., BRADE, L., MAMAT, U. & BRADE, H. (1994). Lipopolysaccharide smooth-rough phase variation in bacteria of the genus Chlamydia. *Infect Immun*, **62,** 2270-6.

LY, H., FRANCONE, O.L., FIELDING, C.J., SHIGENAGA, J.K., MOSER, A.H., GRUNFELD, C. & FEINGOLD, K.R. (1995). Endotoxin and TNF lead to reduced plasma LCAT activity and decreased hepatic LCAT mRNA levels in Syrian hamsters. *J Lipid Res*, **36,** 1254-63.

MAASS, M., BARTELS, C., ENGEL, P.M., MAMAT, U. & SIEVERS, H.H. (1998). Endovascular presence of viable Chlamydia pneumoniae is a common phenomenon in coronary artery disease. *J Am Coll Cardiol*, **31,** 827-32.

MAEJIMA, T., YAMAZAKI, H., AOKI, T., TAMAKI, T., SATO, F., KITAHARA, M. & SAITO, Y. (2004). Effect of pitavastatin on apolipoprotein A-I production in HepG2 cell. *Biochem Biophys Res Commun*, **324,** 835-9.

MALINVERNI, R., KUO, C.C., CAMPBELL, L.A. & GRAYSTON, J.T. (1995). Reactivation of Chlamydia pneumoniae lung infection in mice by cortisone. *J Infect Dis*, **172,** 593-4.

MARAHA, B., DEN HEIJER, M., WULLINK, M., VAN DER ZEE, A., BERGMANS, A., VERBAKEL, H., KERVER, M., GRAAFSMA, S., KRANENDONK, S. & PEETERS, M. (2001). Detection of Chlamydia pneumoniae DNA in buffy-coat samples of patients with abdominal aortic aneurysm. *Eur J Clin Microbiol Infect Dis*, **20,** 111-6.

MASSAMIRI, T., TOBIAS, P.S. & CURTISS, L.K. (1997). Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: role of apolipoprotein A-I. *J Lipid Res*, **38,** 516-25.

MAURIN, M., EB, F., ETIENNE, J. & RAOULT, D. (1997). Serological cross-reactions between Bartonella and Chlamydia species: implications for diagnosis. *J Clin Microbiol*, **35,** 2283-7.

MEANS, T.K., GOLENBOCK, D.T. & FENTON, M.J. (2000). Structure and function of Toll-like receptor proteins. *Life Sci*, **68,** 241-58.

MEIR, K.S. & LEITERSDORF, E. (2004). Atherosclerosis in the apolipoprotein-E-deficient mouse: a decade of progress. *Arterioscler Thromb Vasc Biol*, **24,** 1006-14.

MEMON, R.A., FEINGOLD, K.R., MOSER, A.H., DOERRLER, W., ADI, S., DINARELLO, C.A. & GRUNFELD, C. (1992). Differential effects of interleukin-1 and tumor necrosis factor on ketogenesis. *Am J Physiol*, **263,** E301-9.

MEMON, R.A., GRUNFELD, C., MOSER, A.H. & FEINGOLD, K.R. (1993). Tumor necrosis factor mediates the effects of endotoxin on cholesterol and triglyceride metabolism in mice. *Endocrinology*, **132,** 2246-53.

MEMON, R.A., SHECHTER, I., MOSER, A.H., SHIGENAGA, J.K., GRUNFELD, C. & FEINGOLD, K.R. (1997). Endotoxin, tumor necrosis factor, and interleukin-1 decrease hepatic squalene synthase activity, protein, and mRNA levels in Syrian hamsters. *J Lipid Res*, **38,** 1620-9.

MIYA, N., OGUCHI, S., WATANABE, I. & KANMATSUSE, K. (2004). Relation of secretory phospholipase A(2) and high-sensitivity C-reactive protein to Chlamydia pneumoniae infection in acute coronary syndromes. *Circ J*, **68,** 628-33.

MOAZED, T.C., CAMPBELL, L.A., ROSENFELD, M.E., GRAYSTON, J.T. & KUO, C.C. (1999). Chlamydia pneumoniae infection accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. *J Infect Dis*, **180,** 238-41.

MOAZED, T.C., KUO, C., GRAYSTON, J.T. & CAMPBELL, L.A. (1997). Murine models of Chlamydia pneumoniae infection and atherosclerosis. *J Infect Dis*, **175,** 883-90.

MOAZED, T.C., KUO, C.C., GRAYSTON, J.T. & CAMPBELL, L.A. (1998). Evidence of systemic dissemination of Chlamydia pneumoniae via macrophages in the mouse. *J Infect Dis*, **177,** 1322-5.

MOLESTINA, R.E., MILLER, R.D., LENTSCH, A.B., RAMIREZ, J.A. & SUMMERSGILL, J.T. (2000). Requirement for NF-kappaB in transcriptional activation of monocyte chemotactic protein 1 by Chlamydia pneumoniae in human endothelial cells. *Infect Immun*, **68,** 4282-8.

MORRISON, D.C. & RYAN, J.L. (1987). Endotoxins and disease mechanisms. *Annu Rev Med*, **38,** 417-32.

MORRISON, R.P., BELLAND, R.J., LYNG, K. & CALDWELL, H.D. (1989). Chlamydial disease pathogenesis. The 57-kD chlamydial hypersensitivity antigen is a stress response protein. *J Exp Med*, **170,** 1271-83.

MOSORIN, M., SURCEL, H.M., LAURILA, A., LEHTINEN, M., KARTTUNEN, R., JUVONEN, J., PAAVONEN, J., MORRISON, R.P., SAIKKU, P. & JUVONEN, T. (2000). Detection of Chlamydia pneumoniae-reactive T lymphocytes in human atherosclerotic plaques of carotid artery. *Arterioscler Thromb Vasc Biol*, **20,** 1061-7.

MOULDER, J., HATCH, T., KUO, C., SCACHTER, J. & STORZ, J. (1984). *Genus Chlamydia*. Baltimore: The Williams and Wilkins Co.

MUHLESTEIN, J.B., HAMMOND, E.H., CARLQUIST, J.F., RADICKE, E., THOMSON, M.J., KARAGOUNIS, L.A., WOODS, M.L. & ANDERSON, J.L. (1996). Increased incidence of Chlamydia species within the coronary arteries of patients with symptomatic atherosclerotic versus other forms of cardiovascular disease. *J Am Coll Cardiol*, **27,** 1555-61.

MUKERJEE, P., KASTOWSKY, M., OBST, S. & TAKAYAMA, K. (1999). In *Endotoxin in health and disease*. eds Brade, H., Morrison, D., Opal, S. & Vogel, S. pp. 221-228. New York: Marcel Dekker Inc.

MULLER-LOENNIES, S., GRONOW, S., BRADE, L., MACKENZIE, R., KOSMA, P. & BRADE, H. (2005). A Monoclonal Antibody against a Carbohydrate Epitope in Lipopolysaccharide Differentiates Chlamydophila psittaci from Chlamydophila pecorum, Chlamydophila pneumoniae and Chlamydia trachomatis. *Glycobiology*.

MURRAY, L.J., O'REILLY, D.P., ONG, G.M., O'NEILL, C., EVANS, A.E. & BAMFORD, K.B. (1999). Chlamydia pneumoniae antibodies are associated with an atherogenic lipid profile. *Heart*, **81,** 239-44.

NAGAI, Y., AKASHI, S., NAGAFUKU, M., OGATA, M., IWAKURA, Y., AKIRA, S., KITAMURA, T., KOSUGI, A., KIMOTO, M. & MIYAKE, K. (2002). Essential role of MD-2 in LPS responsiveness and TLR4 distribution. *Nat Immunol*, **3,** 667-72.

NAKASHIMA, Y., PLUMP, A.S., RAINES, E.W., BRESLOW, J.L. & ROSS, R. (1994). ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler Thromb*, **14,** 133-40.

NETEA, M.G., DEMACKER, P.N., KULLBERG, B.J., JACOBS, L.E., VERVER-JANSEN, T.J., BOERMAN, O.C., STALENHOEF, A.F. & VAN DER MEER, J.W. (1998). Bacterial lipopolysaccharide binds and stimulates cytokine-producing cells before neutralization by endogenous lipoproteins can occur. *Cytokine*, **10,** 766-72.

NETEA, M.G., KULLBERG, B.J., GALAMA, J.M., STALENHOEF, A.F., DINARELLO, C.A. & VAN DER MEER, J.W. (2002). Non-LPS components of Chlamydia pneumoniae stimulate cytokine production through Toll-like receptor 2-dependent pathways. *Eur J Immunol*, **32,** 1188-95.

NEUGEBAUER, J.M. (1990). Detergents: an overview. *Methods Enzymol*, **182,** 239-53.

NILSSON-EHLE, P., GARFINKEL, A.S. & SCHOTZ, M.C. (1980). Lipolytic enzymes and plasma lipoprotein metabolism. *Annu Rev Biochem*, **49,** 667-93.

NISHIDA, H.I., ARAI, H. & NISHIDA, T. (1993). Cholesterol ester transfer mediated by lipid transfer protein as influenced by changes in the charge characteristics of plasma lipoproteins. *J Biol Chem*, **268,** 16352-60.

NURMINEN, M., LEINONEN, M., SAIKKU, P. & MÄKELÄ, P.H. (1983). The genus-specific antigen of Chlamydia: resemblance to the lipopolysaccharide of enteric bacteria. *Science*, **220,** 1279-81.

NURMINEN, M., RIETSCHEL, E.T. & BRADE, H. (1985). Chemical characterization of Chlamydia trachomatis lipopolysaccharide. *Infect Immun*, **48,** 573-5.

NURMINEN, M., WAHLSTRÖM, E., KLEEMOLA, M., LEINONEN, M., SAIKKU, P. & MÄKELÄ, P.H. (1984). Immunologically related ketodeoxyoctonate-containing structures in Chlamydia trachomatis, Re mutants of Salmonella species, and Acinetobacter calcoaceticus var. anitratus. *Infect Immun*, **44,** 609-13.

ONG, G., THOMAS, B.J., MANSFIELD, A.O., DAVIDSON, B.R. & TAYLOR-ROBINSON, D. (1996). Detection and widespread distribution of Chlamydia pneumoniae in the vascular system and its possible implications. *J Clin Pathol*, **49,** 102-6.

OPAL, S.M., SCANNON, P.J., VINCENT, J.L., WHITE, M., CARROLL, S.F., PALARDY, J.E., PAREJO, N.A., PRIBBLE, J.P. & LEMKE, J.H. (1999). Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock. *J Infect Dis*, **180,** 1584-9.

ORIEL, J. (1994). The History of Chlamydial Infections. In *Chlamydial Infections. Proceedings of the Eighth International Symposium on Human Chlamydial Infections.* eds Orfila, J., Byrne, G.I., Chernesky, M.A., Grayston, J.T., Jones, R.B., Ridgway, G.L., Saikku, P., Schachter, J., Stamm, W.E. & Stephens, R.S. pp. 3-6. Gouvieux-Chantilly: Societá Editrice Esculapio.

PACKARD, C.J. & SHEPHERD, J. (1999). Physiology of the lipoprotein transport system: an overview of lipoprotein metabolism. In *Lipoproteins in health and disease*. eds Betteridge, D.J., Illingworth, D.R. & Shepherd, J. pp. 17-30. London: Arnold.

PAIGEN, B., ISHIDA, B.Y., VERSTUYFT, J., WINTERS, R.B. & ALBEE, D. (1990). Atherosclerosis susceptibility differences among progenitors of recombinant inbred strains of mice. *Arteriosclerosis*, **10,** 316-23.

PAIGEN, B., MITCHELL, D., REUE, K., MORROW, A., LUSIS, A.J. & LEBOEUF, R.C. (1987). Ath-1, a gene determining atherosclerosis susceptibility and high density lipoprotein levels in mice. *Proc Natl Acad Sci U S A*, **84,** 3763-7.

PAIGEN, B., MORROW, A., BRANDON, C., MITCHELL, D. & HOLMES, P. (1985). Variation in susceptibility to atherosclerosis among inbred strains of mice. *Atherosclerosis*, **57,** 65-73.

PALDANIUS, M., BLOIGU, A., ALHO, M., LEINONEN, M. & SAIKKU, P. (2005). Prevalence and persistence of Chlamydia pneumoniae antibodies in healthy laboratory personnel in Finland. *Clin Diagn Lab Immunol*, **12,** 654-9.

PARRILLO, J.E. (1993). Pathogenetic mechanisms of septic shock. *N Engl J Med*, **328,** 1471-7.

PEDERSEN, A.S., CHRISTIANSEN, G. & BIRKELUND, S. (2001). Differential expression of Pmp10 in cell culture infected with Chlamydia pneumoniae CWL029. *FEMS Microbiol Lett*, **203,** 153-9.

PEDERSEN, T.R. (1994). Randomised trial of cholesterol lowering in 4444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). *Lancet*, **344,** 1383-9.

PEDERSEN, T.R., OLSSON, A.G., FAERGEMAN, O., KJEKSHUS, J., WEDEL, H., BERG, K., WILHELMSEN, L., HAGHFELT, T., THORGEIRSSON, G., PYÖRÄLÄ, K., MIETTINEN, T., CHRISTOPHERSEN, B., TOBERT, J.A., MUSLINER, T.A. & COOK, T.J. (1998). Lipoprotein changes and reduction in the incidence of major coronary heart disease events in the Scandinavian Simvastatin Survival Study (4S). *Circulation*, **97,** 1453-60.

PEELING, R., WANG, S., GRAYSTON, J., F, BOMAN, J., CLAD, A. & AL., E. (1998). Chlamydia serology: inter-laboratory variation in microimmunofluorescence results. In *Chlamydial infections. Proceedings of the 9th International Symposium on Human Chlamydial Infections*. eds Stephens, R.S., Byrne, G.I. & al., e. pp. 159-162. Napa.

PENTTILÄ, J.M., ANTTILA, M., PUOLAKKAINEN, M., LAURILA, A., VARKILA, K., SARVAS, M., MÄKELÄ, P.H. & RAUTONEN, N. (1998). Local immune responses to Chlamydia pneumoniae in the lungs of BALB/c mice during primary infection and reinfection. *Infect Immun*, **66,** 5113-8.

PENTTILÄ, J.M., ANTTILA, M., VARKILA, K., PUOLAKKAINEN, M., SARVAS, M., MÄKELÄ, P.H. & RAUTONEN, N. (1999). Depletion of CD8+ cells abolishes memory in acquired immunity against Chlamydia pneumoniae in BALB/c mice. *Immunology*, **97,** 490-6.

PEREZ MELGOSA, M., KUO, C.C. & CAMPBELL, L.A. (1991). Sequence analysis of the major outer membrane protein gene of Chlamydia pneumoniae. *Infect Immun*, **59,** 2195-9.

PIERRI, H., HIGUCHI-DOS-SANTOS, M.H., HIGUCHI, M.D., PALOMINO, S., SAMBIASE, N.V., DEMARCHI, L.M., RODRIGUES, G.H., NUSSBACHER, A., RAMIRES, J.A. & WAJNGARTEN, M. (2005). Density of Chlamydia pneumoniae is increased in fibrotic and calcified areas of degenerative aortic stenosis. *Int J Cardiol*.

PLUMP, A.S., SMITH, J.D., HAYEK, T., AALTO-SETALA, K., WALSH, A., VERSTUYFT, J.G., RUBIN, E.M. & BRESLOW, J.L. (1992). Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell*, **71,** 343-53.

POPPERT, S., SCHLAUPITZ, K., MARRE, R., VOISARD, R., ROESSLER, W., WECKERMANN, D., WEINGARTNER, K. & ESSIG, A. (2005). Chlamydia pneumoniae in an ex vivo human artery culture model. *Atherosclerosis*.

PREBECK, S., BRADE, H., KIRSCHNING, C.J., DA COSTA, C.P., DURR, S., WAGNER, H. & MIETHKE, T. (2003). The Gram-negative bacterium Chlamydia trachomatis L2 stimulates tumor necrosis factor secretion by innate immune cells independently of its endotoxin. *Microbes Infect*, **5,** 463-70.

PUOLAKKAINEN, M., LINNANMÄKI, E., PAAVONEN, J., LEINONEN, M. & SAIKKU, P. (1990). Chlamydial lipopolysaccharide containing circulating immune complexes in pelvic inflammatory disease. In *Chlamydial infections*. ed Bowie WR, C.H., Jones RP, Mårdh PA, Ridgway GL, Schachter J, Stamm WE, Ward ME. pp. 319-322. Cambridge: Cambridge University Press.

QURESHI, N., KALTASHOV, I., WALKER, K., DOROSHENKO, V., COTTER, R.J., TAKAYAMA, K., SIEVERT, T.R., RICE, P.A., LIN, J.S. & GOLENBOCK, D.T. (1997). Structure of the monophosphoryl lipid A moiety obtained from the lipopolysaccharide of Chlamydia trachomatis. *J Biol Chem*, **272,** 10594-600.

RAETZ, C.R. (1990). Biochemistry of endotoxins. *Annu Rev Biochem*, **59,** 129-70.

RAMADORI, G., MEYER ZUM BUSCHENFELDE, K.H., TOBIAS, P.S., MATHISON, J.C. & ULEVITCH, R.J. (1990). Biosynthesis of lipopolysaccharide-binding protein in rabbit hepatocytes. *Pathobiology*, **58,** 89-94.

RAMIREZ, J.A. (1996). Isolation of Chlamydia pneumoniae from the coronary artery of a patient with coronary atherosclerosis. The Chlamydia pneumoniae/Atherosclerosis Study Group. *Ann Intern Med*, **125,** 979-82.

RAULSTON, J.E., DAVIS, C.H., SCHMIEL, D.H., MORGAN, M.W. & WYRICK, P.B. (1993). Molecular characterization and outer membrane association of a Chlamydia trachomatis protein related to the hsp70 family of proteins. *J Biol Chem*, **268,** 23139-47.

READ, T.E., HARRIS, H.W., GRUNFELD, C., FEINGOLD, K.R., KANE, J.P. & RAPP, J.H. (1993). The protective effect of serum lipoproteins against bacterial lipopolysaccharide. *Eur Heart J*, **14 Suppl K,** 125-9.

REDDICK, R.L., ZHANG, S.H. & MAEDA, N. (1994). Atherosclerosis in mice lacking apo E. Evaluation of lesional development and progression. *Arterioscler Thromb*, **14,** 141-7.

RIDKER, P.M. (2003). Rosuvastatin in the primary prevention of cardiovascular disease among patients with low levels of low-density lipoprotein cholesterol and elevated high-sensitivity C-reactive protein: rationale and design of the JUPITER trial. *Circulation*, **108,** 2292-7.

RIDKER, P.M., RIFAI, N., CLEARFIELD, M., DOWNS, J.R., WEIS, S.E., MILES, J.S. & GOTTO, A.M., JR. (2001). Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med*, **344,** 1959-65.

RIETSCHEL, E.T. & BRADE, H. (1992). Bacterial endotoxins. *Sci Am*, **267,** 54-61.

RIETSCHEL, E.T., BRADE, H., HOLST, O., BRADE, L., MULLER-LOENNIES, S., MAMAT, U., ZAHRINGER, U., BECKMANN, F., SEYDEL, U., BRANDENBURG, K., ULMER, A.J., MATTERN, T., HEINE, H., SCHLETTER, J., LOPPNOW, H., SCHONBECK, U., FLAD, H.D., HAUSCHILDT, S., SCHADE, U.F., DI PADOVA, F., KUSUMOTO, S. & SCHUMANN, R.R. (1996). Bacterial endotoxin: Chemical constitution, biological

recognition, host response, and immunological detoxification. *Curr Top Microbiol Immunol*, **216,** 39-81.

RIETSCHEL, E.T., KIRIKAE, T., SCHADE, F.U., MAMAT, U., SCHMIDT, G., LOPPNOW, H., ULMER, A.J., ZAHRINGER, U., SEYDEL, U., DI PADOVA, F. & ET AL. (1994). Bacterial endotoxin: molecular relationships of structure to activity and function. *Faseb J*, **8,** 217-25.

ROBLIN, P.M., DUMORNAY, W. & HAMMERSCHLAG, M.R. (1992). Use of HEp-2 cells for improved isolation and passage of Chlamydia pneumoniae. *J Clin Microbiol*, **30,** 1968-71.

ROCKEY, D.D., HEINZEN, R.A. & HACKSTADT, T. (1995). Cloning and characterization of a Chlamydia psittaci gene coding for a protein localized in the inclusion membrane of infected cells. *Mol Microbiol*, **15,** 617-26.

ROSS, R. (1999). Atherosclerosis is an inflammatory disease. *Am Heart J*, **138,** S419-20.

ROTHSTEIN, N.M., QUINN, T.C., MADICO, G., GAYDOS, C.A. & LOWENSTEIN, C.J. (2001). Effect of azithromycin on murine arteriosclerosis exacerbated by Chlamydia pneumoniae. *J Infect Dis*, **183,** 232-238.

ROTTENBERG, M.E., GIGLIOTTI-ROTHFUCHS, A. & WIGZELL, H. (2002). The role of IFN-gamma in the outcome of chlamydial infection. *Curr Opin Immunol*, **14,** 444-51.

RUND, S., LINDNER, B., BRADE, H. & HOLST, O. (1999). Structural analysis of the lipopolysaccharide from Chlamydia trachomatis serotype L2. *J Biol Chem*, **274,** 16819-24.

RUND, S., LINDNER, B., BRADE, H. & HOLST, O. (2000). Structural analysis of the lipopolysaccharide from Chlamydophila psittaci strain 6BC. *Eur J Biochem*, **267,** 5717-26.

SAIKKU, P. (1994). Diagnosis of acute and chronic *Chlamydia pneumoniae* infections. In *Chlamydial infections. Proceedings of the Eighth International Symposium on Human Chlamydial Infections*. eds Orfila, J., Byrne, G.I., Chernesky, M.A., Grayston, J.T., Jones, R.B., Ridgeway, G.L., Saikku, P., Schachter, J., Stamm, W.E. & Stephens, R.S. pp. 163-172. Gouvieux-Chantilly: Societá Editrice Esculapio.

SAIKKU, P. (1992). The epidemiology and significance of Chlamydia pneumoniae. *J Infect*, **25 Suppl 1,** 27-34.

SAIKKU, P., LEINONEN, M., MATTILA, K., EKMAN, M.R., NIEMINEN, M.S., MÄKELÄ, P.H., HUTTUNEN, J.K. & VALTONEN, V. (1988). Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet*, **2,** 983-6.

SAIKKU, P., LEINONEN, M., TENKANEN, L., LINNANMÄKI, E., EKMAN, M.R., MANNINEN, V., MÄNTTÄRI, M., FRICK, M.H. & HUTTUNEN, J.K. (1992). Chronic Chlamydia pneumoniae infection as a risk factor for coronary heart disease in the Helsinki Heart Study. *Ann Intern Med*, **116,** 273-8.

SAIKKU, P., WANG, S.P., KLEEMOLA, M., BRANDER, E., RUSANEN, E. & GRAYSTON, J.T. (1985). An epidemic of mild pneumonia due to an unusual strain of Chlamydia psittaci. *J Infect Dis*, **151,** 832-9.

SAKAGUCHI, O. & SAKAGUCHI, S. (1979). Alterations of lipid metabolism in mice injected with endotoxin. *Microbiol Immunol*, **23,** 71-85.

SAMMALKORPI, K., VALTONEN, V., KERTTULA, Y., NIKKILÄ, E. & TASKINEN, M.R. (1988). Changes in serum lipoprotein pattern induced by acute infections. *Metabolism*, **37,** 859-65.

SÄVYKOSKI, T., HARJU, T., PALDANIUS, M., KUITUNEN, H., BLOIGU, A., WAHLSTRÖM, E., RYTILÄ, P., KINNULA, V., SAIKKU, P. & LEINONEN, M. (2004). Chlamydia pneumoniae infection and inflammation in adults with asthma. *Respiration*, **71,** 120-5.

SCHACHTER, J., STEPHENS, R.S., TIMMS, P., KUO, C., BAVOIL, P.M., BIRKELUND, S., BOMAN, J., CALDWELL, H., CAMPBELL, L.A., CHERNESKY, M., CHRISTIANSEN, G., CLARKE, I.N., GAYDOS, C., GRAYSTON, J.T., HACKSTADT, T., HSIA, R., KALTENBOECK, B., LEINONEN, M., OCJIUS, D., MCCLARTY, G., ORFILA, J., PEELING, R., PUOLAKKAINEN, M., QUINN, T.C., RANK, R.G., RAULSTON, J., RIDGEWAY, G.L., SAIKKU, P., STAMM, W.E., TAYLOR-ROBINSON, D.T., WANG, S.P. & WYRICK, P.B. (2001). Radical changes to chlamydial taxonomy are not necessary just yet. *Int J Syst Evol Microbiol*, **51,** 249, 251-3.

SCHILDKRAUT, J.M., MYERS, R.H., CUPPLES, L.A., KIELY, D.K. & KANNEL, W.B. (1989). Coronary risk associated with age and sex of parental heart disease in the Framingham Study. *Am J Cardiol*, **64,** 555-9.

SCHOLL, R.A., LANG, C.H. & BAGBY, G.J. (1984). Hypertriglyceridemia and its relation to tissue lipoprotein lipase activity in endotoxemic, Escherichia coli bacteremic, and polymicrobial septic rats. *J Surg Res*, **37,** 394-401.

SCHUMANN, R.R., KIRSCHNING, C.J., UNBEHAUN, A., ABERLE, H.P., KNOPE, H.P., LAMPING, N., ULEVITCH, R.J. & HERRMANN, F. (1996). The lipopolysaccharide-binding protein is a secretory class 1 acute-phase protein whose gene is transcriptionally activated by APRF/STAT/3 and other cytokine-inducible nuclear proteins. *Mol Cell Biol*, **16,** 3490-503.

SCHUMANN, R.R. & ZWEIGNER, J. (1999). A novel acute-phase marker: lipopolysaccharide binding protein (LBP). *Clin Chem Lab Med*, **37,** 271-4.

SCIDMORE-CARLSON, M.A., SHAW, E.I., DOOLEY, C.A., FISCHER, E.R. & HACKSTADT, T. (1999). Identification and characterization of a Chlamydia trachomatis early operon encoding four novel inclusion membrane proteins. *Mol Microbiol*, **33,** 753-65.

SESSA, R., DI PIETRO, M., SCHIAVONI, G., SANTINO, I., CIPRIANI, P., ROMANO, S., PENCO, M. & DEL PIANO, M. (2001). Prevalence of Chlamydia pneumoniae in peripheral blood mononuclear cells in Italian patients with acute ischaemic heart disease. *Atherosclerosis*, **159,** 521-5.

SEYDEL, U. (1998). Molecular basis of endotoxin toxicity. *Ann N Y Acad Sci*, **851,** 406-7.

SHEPHERD, J., COBBE, S.M., FORD, I., ISLES, C.G., LORIMER, A.R., MACFARLANE, P.W., MCKILLOP, J.H. & PACKARD, C.J. (1995). Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. West of Scotland Coronary Prevention Study Group. *N Engl J Med*, **333,** 1301-7.

SHIMAZU, R., AKASHI, S., OGATA, H., NAGAI, Y., FUKUDOME, K., MIYAKE, K. & KIMOTO, M. (1999). MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *J Exp Med*, **189,** 1777-82.

SHOR, A., KUO, C.C. & PATTON, D.L. (1992). Detection of Chlamydia pneumoniae in coronary arterial fatty streaks and atheromatous plaques. *S Afr Med J*, **82,** 158-61.

SINISALO, J., MATTILA, K., VALTONEN, V., ANTTONEN, O., JUVONEN, J., MELIN, J., VUORINEN-MARKKOLA, H. & NIEMINEN, M.S. (2002). Effect of 3 months of antimicrobial treatment with clarithromycin in acute non-q-wave coronary syndrome. *Circulation*, **105,** 1555-60.

SMADEL, J. (1943). Atypical pneumonia and psittacosis. *J Clin Invest*, **22,** 57-65.

SMIEJA, M., CHONG, S., NATARAJAN, M., PETRICH, A., RAINEN, L. & MAHONY, J.B. (2001). Circulating nucleic acids of Chlamydia pneumoniae and cytomegalovirus in patients undergoing coronary angiography. *J Clin Microbiol*, **39,** 596-600.

SMIEJA, M., MAHONY, J., PETRICH, A., BOMAN, J. & CHERNESKY, M. (2002). Association of circulating Chlamydia pneumoniae DNA with cardiovascular disease: a systematic review. *BMC Infect Dis*, **2,** 21.

SPRIGGS, D.R., SHERMAN, M.L., MICHIE, H., ARTHUR, K.A., IMAMURA, K., WILMORE, D., FREI, E., 3RD & KUFE, D.W. (1988). Recombinant human tumor necrosis factor administered as a 24-hour intravenous infusion. A phase I and pharmacologic study. *J Natl Cancer Inst*, **80,** 1039-44.

STAFFORINI, D.M., MCINTYRE, T.M., ZIMMERMAN, G.A. & PRESCOTT, S.M. (1997). Platelet-activating factor acetylhydrolases. *J Biol Chem*, **272,** 17895-8.

STARY, H.C., CHANDLER, A.B., DINSMORE, R.E., FUSTER, V., GLAGOV, S., INSULL, W., JR., ROSENFELD, M.E., SCHWARTZ, C.J., WAGNER, W.D. & WISSLER, R.W. (1995). A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation*, **92,** 1355-74.

STEIN, E.A., LANE, M. & LASKARZEWSKI, P. (1998). Comparison of statins in hypertriglyceridemia. *Am J Cardiol*, **81,** 66B-69B.

STEPHENS, R.S., KALMAN, S., LAMMEL, C., FAN, J., MARATHE, R., ARAVIND, L., MITCHELL, W., OLINGER, L., TATUSOV, R.L., ZHAO, Q., KOONIN, E.V. & DAVIS, R.W. (1998). Genome sequence of an obligate intracellular pathogen of humans: Chlamydia trachomatis. *Science*, **282,** 754-9.

STEPHENS, R.S., WAGAR, E.A. & SCHOOLNIK, G.K. (1988). High-resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of Chlamydia trachomatis. *J Exp Med*, **167,** 817-31.

STOLL, L.L., DENNING, G.M., LI, W.G., RICE, J.B., HARRELSON, A.L., ROMIG, S.A., GUNNLAUGSSON, S.T., MILLER, F.J., JR. & WEINTRAUB, N.L. (2004). Regulation of endotoxin-induced proinflammatory activation in human coronary artery cells: expression of functional membrane-bound CD14 by human coronary artery smooth muscle cells. *J Immunol*, **173,** 1336-43.

STORZ, J. & SPEARS, P. (1977). Chlamydiales: properties, cycle of development and effect on eukaryotic host cells. *Curr Top Microbiol Immunol*, **76,** 167-214.

STREJA, L., PACKARD, C.J., SHEPHERD, J., COBBE, S. & FORD, I. (2002). Factors affecting low-density lipoprotein and high-density lipoprotein cholesterol response to pravastatin in the West Of Scotland Coronary Prevention Study (WOSCOPS). *Am J Cardiol*, **90,** 731-6.

SUBTIL, A., BLOCKER, A. & DAUTRY-VARSAT, A. (2000). Type III secretion system in Chlamydia species: identified members and candidates. *Microbes Infect*, **2,** 367-9.

SUBTIL, A. & DAUTRY-VARSAT, A. (2004). Chlamydia: five years A.G. (after genome). *Curr Opin Microbiol*, **7,** 85-92.

SUBTIL, A., DELEVOYE, C., BALANA, M.E., TASTEVIN, L., PERRINET, S. & DAUTRY-VARSAT, A. (2005). A directed screen for chlamydial proteins secreted by a type III mechanism identifies a translocated protein and numerous other new candidates. *Mol Microbiol*, **56,** 1636-47.

TALL, A.R. (1993). Plasma cholesteryl ester transfer protein. *J Lipid Res*, **34,** 1255-74.

TAPPING, R.I. & TOBIAS, P.S. (2000). Soluble CD14-mediated cellular responses to lipopolysaccharide. *Chem Immunol*, **74,** 108-21.

THOM, D.H., GRAYSTON, J.T., WANG, S.P., KUO, C.C. & ALTMAN, J. (1990). Chlamydia pneumoniae strain TWAR, Mycoplasma pneumoniae, and viral infections in acute respiratory disease in a university student health clinic population. *Am J Epidemiol*, **132,** 248-56.

TOLLEFSON, J.H., RAVNIK, S. & ALBERS, J.J. (1988). Isolation and characterization of a phospholipid transfer protein (LTP-II) from human plasma. *J Lipid Res*, **29,** 1593-602.

TÖRMÄKANGAS, L., ALAKÄRPPÄ, H., DAVID, D.B., LEINONEN, M. & SAIKKU, P. (2004). Telithromycin treatment of chronic Chlamydia pneumoniae infection in C57BL/6J mice. *Antimicrob Agents Chemother*, **48,** 3655-61.

TRIPP, R.J., TABARES, A., WANG, H. & LANZA-JACOBY, S. (1993). Altered hepatic production of apolipoproteins B and E in the fasted septic rat: factors in the development of hypertriglyceridemia. *J Surg Res*, **55,** 465-72.

ULEVITCH, R.J. & JOHNSTON, A.R. (1978). The modification of biophysical and endotoxic properties of bacterial lipopolysaccharides by serum. *J Clin Invest*, **62,** 1313-24.

ULEVITCH, R.J., JOHNSTON, A.R. & WEINSTEIN, D.B. (1979). New function for high density lipoproteins. Their participation in intravascular reactions of bacterial lipopolysaccharides. *J Clin Invest*, **64,** 1516-24.

ULEVITCH, R.J. & TOBIAS, P.S. (1995). Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol*, **13,** 437-57.

UREN, N.G., CAMICI, P.G., MELIN, J.A., BOL, A., DE BRUYNE, B., RADVAN, J., OLIVOTTO, I., ROSEN, S.D., IMPALLOMENI, M. & WIJNS, W. (1995). Effect of aging on myocardial perfusion reserve. *J Nucl Med*, **36,** 2032-6.

VAN LEEUWEN, H.J., HEEZIUS, E.C., DALLINGA, G.M., VAN STRIJP, J.A., VERHOEF, J. & VAN KESSEL, K.P. (2003). Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med*, **31,** 1359-66.

VAN LENTEN, B.J., FOGELMAN, A.M., HABERLAND, M.E. & EDWARDS, P.A. (1986). The role of lipoproteins and receptor-mediated endocytosis in the transport of bacterial lipopolysaccharide. *Proc Natl Acad Sci U S A*, **83,** 2704-8.

VAN LENTEN, B.J., HAMA, S.Y., DE BEER, F.C., STAFFORINI, D.M., MCINTYRE, T.M., PRESCOTT, S.M., LA DU, B.N., FOGELMAN, A.M. & NAVAB, M. (1995). Anti-inflammatory HDL becomes pro-inflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J Clin Invest*, **96,** 2758-67.

VAN LENTEN, B.J., WAGNER, A.C., NAYAK, D.P., HAMA, S., NAVAB, M. & FOGELMAN, A.M. (2001). High-density lipoprotein loses its anti-inflammatory properties during acute influenza a infection. *Circulation*, **103,** 2283-8.

WAN, Y., FREESWICK, P.D., KHEMLANI, L.S., KISPERT, P.H., WANG, S.C., SU, G.L. & BILLIAR, T.R. (1995). Role of lipopolysaccharide (LPS), interleukin-1, interleukin-6, tumor necrosis factor, and dexamethasone in regulation of LPS-binding protein expression in normal hepatocytes and hepatocytes from LPS-treated rats. *Infect Immun*, **63,** 2435-42.

WANG, S.P. & GRAYSTON, J.T. (1991). Chlamydia pneumoniae elementary body antigenic reactivity with fluorescent antibody is destroyed by methanol. *J Clin Microbiol*, **29,** 1539-41.

WANG, S.P. & GRAYSTON, J.T. (1970). Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. *Am J Ophthalmol*, **70,** 367-74.

VANROMPAY, D., CHARLIER, G., DUCATELLE, R. & HAESEBROUCK, F. (1996). Ultrastructural changes in avian Chlamydia psittaci serovar A-, B-, and D-infected Buffalo Green Monkey cells. *Infect Immun*, **64,** 1265-71.

WARAVDEKAR, V.S. & SASLAW, L.D. (1959). A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde-thiobarbituric acid reaction. *J Biol Chem*, **234,** 1945-1950.

WARD, M.E. (1983). Chlamydial classification, development and structure. *Br Med Bull*, **39,** 109-15.

VAUGHAN, C.J. & GOTTO, A.M., JR. (2004). Update on statins: 2003. *Circulation*, **110,** 886-92.

VEGA, G.L., WEINER, M.F., LIPTON, A.M., VON BERGMANN, K., LUTJOHANN, D., MOORE, C. & SVETLIK, D. (2003). Reduction in levels of 24S-hydroxycholesterol by statin treatment in patients with Alzheimer disease. *Arch Neurol*, **60,** 510-5.

VERGANI, C., TROVATO, G., DELU, A., PIETROGRANDE, M. & DIOGUARDI, N. (1978). Serum total lipids, lipoprotein cholesterol, and apolipoprotein A in acute viral hepatitis and chronic liver disease. *J Clin Pathol*, **31,** 772-8.

VESY, C.J., KITCHENS, R.L., WOLFBAUER, G., ALBERS, J.J. & MUNFORD, R.S. (2000). Lipopolysaccharide-binding protein and phospholipid transfer protein release lipopolysaccharides from gram-negative bacterial membranes. *Infect Immun*, **68,** 2410-7.

WICK, G., PERSCHINKA, H. & MILLONIG, G. (2001). Atherosclerosis as an autoimmune disease: an update. *Trends Immunol*, **22,** 665-9.

VICTOROV, A.V., MEDVEDEVA, N.V., GLADKAYA, E.M., MOROZKIN, A.D., PODREZ, E.A., KOSYKH, V.A. & YURKIV, V.A. (1989). Composition and structure of lipopolysaccharide-human plasma low density lipoprotein complex. Analytical ultracentrifugation, 31P-NMR, ESR and fluorescence spectroscopy studies. *Biochim Biophys Acta*, **984,** 119-27.

VIRIYAKOSOL, S. & KIRKLAND, T.N. (1996). The N-terminal half of membrane CD14 is a functional cellular lipopolysaccharide receptor. *Infect Immun*, **64,** 653-6.

VIRIYAKOSOL, S., TOBIAS, P.S., KITCHENS, R.L. & KIRKLAND, T.N. (2001). MD-2 binds to bacterial lipopolysaccharide. *J Biol Chem*, **276,** 38044-51.

WOLF, K., FISCHER, E., MEAD, D., ZHONG, G., PEELING, R., WHITMIRE, B. & CALDWELL, H.D. (2001). Chlamydia pneumoniae major outer membrane protein is a surface-exposed antigen that elicits antibodies primarily directed against conformation-dependent determinants. *Infect Immun*, **69,** 3082-91.

VON HERTZEN, L., ALAKARPPA, H., KOSKINEN, R., LIIPPO, K., SURCEL, H.M., LEINONEN, M. & SAIKKU, P. (1997). Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease. *Epidemiol Infect*, **118,** 155-64.

WONG, Y.K., DAWKINS, K.D. & WARD, M.E. (1999). Circulating Chlamydia pneumoniae DNA as a predictor of coronary artery disease. *J Am Coll Cardiol*, **34,** 1435-9.

VREUGDENHIL, A.C., SNOEK, A.M., VAN 'T VEER, C., GREVE, J.W. & BUURMAN, W.A. (2001). LPS-binding protein circulates in association with apoB-containing lipoproteins and enhances endotoxin-LDL/VLDL interaction. *J Clin Invest*, **107,** 225-34.

WURFEL, M.M., HAILMAN, E. & WRIGHT, S.D. (1995). Soluble CD14 acts as a shuttle in the neutralization of lipopolysaccharide (LPS) by LPS-binding protein and reconstituted high density lipoprotein. *J Exp Med*, **181,** 1743-54.

WURFEL, M.M., KUNITAKE, S.T., LICHENSTEIN, H., KANE, J.P. & WRIGHT, S.D. (1994). Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J Exp Med*, **180,** 1025-35.

YANG, Z.P., KUO, C.C. & GRAYSTON, J.T. (1993). A mouse model of Chlamydia pneumoniae strain TWAR pneumonitis. *Infect Immun*, **61,** 2037-40.

ZAHRINGER, U., LINDNER, B. & RIETSCHEL, E.T. (1994). Molecular structure of lipid A, the endotoxic center of bacterial lipopolysaccharides. *Adv Carbohydr Chem Biochem*, **50,** 211-76.

ZHANG, Y.X., STEWART, S.J. & CALDWELL, H.D. (1989). Protective monoclonal antibodies to Chlamydia trachomatis serovar- and serogroup-specific major outer membrane protein determinants. *Infect Immun*, **57,** 636-8.

ZIEGLER, K. & STUNKEL, W. (1992). Tissue-selective action of pravastatin due to hepatocellular uptake via a sodium-independent bile acid transporter. *Biochim Biophys Acta*, **1139,** 203-9.